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Synthesis of New Oxopyrimidine and Cyanopyridine Derivatives Containing the Pyrimidine Benzothiazole Moiety with the Evaluating their Colon Anticancer Bioactivity

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Abstract

In this contribution, new derivatives of bicyclic fused rings with bridgehead nitrogen of 3-substituted imidazo/pyrimidine derivatives A1-A3 were synthesized by reacting 2-aminobenzothiazol, acetyl acetone, and different substituted aldehydes in a one-pot reaction. These compounds A1-A3 were then condensed with 4-bromobenzaldehyde and 4-(*N*,*N*-dimethylamino)benzaldehyde to form new chalcone derivatives A4-A9. Ring closure of these compounds A4-A9 with urea and malononitrile afforded new oxopyrimidine derivatives A10-A15 and cyanopyridine derivatives A16-A21, respectively. These compounds were characterized by FT-IR, ^{1}H NMR, and ^{13}C NMR spectroscopy. The second part of this work included some of the important applications conducted for these compounds to evaluate their biological activities against colon cancer, which showed excellent results against this disease.

Keywords: Imidazo, Pyrimidine, Chalcone, Oxopyrimidine, Cyanopyridine, Colon anticancer, CaCo-2.

تحضير مشتقات جديدة اوكسو بيرميدين و سيانو بريدين تحتوي بيرميدين بنزوثايازول مع تقيم الفعالية البايلوجية ضد سرطان القولون

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الخلاصة

في هذه المساهمة تم تحضير مركبات ثنائية الحلقة الملتحمة مع ذرة نيتروجين جسريه لمشتقات الاميدازو/بيرميدين المعوضه في موقع 3 A1–A3 من تفاعل 2–امينو بنزوثايازول مع الاسيتايل اسيتون ومشتقات الالديهايدات المختلفة في دورق واحد. بعدها ادخلت المركبات A1–A3 في تفاعلات تكثيفية مع 4–برومو بنزلديهايد و 4–داي مثيل امينو بنزلديهايد لتحضير مشتقات الجالكون الجديدة A4–A9، وهذه الجالكونات الجديدة خضعت للغلق الحلقي مع اليوريا مرة و المالونونايترايل مرة اخرى لتخليق مشتقات الاوكسو بيرميدين جديدة A10–A15 ومشتقات السيانو بريدين جديدة A16–A21، على التوالي. شخصت المركبات المحضرة باستخدام مطيافية الاشعة تحت الحمراء ومطيافية الرنين النووي المغناطيسي. الجزء الثاني من العمل تضمن قياس الفعالية البيولوجية لبعض المركبات المحضرة ضد سرطان القولون واظهر نتائج جيدة ضد هذا المرض.

1. Introduction

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Multicomponent reactions (MCR) are one of the most important and efficient reactions in chemical synthesis. MCR involves reacting three or more compounds or starting materials together in one reaction (one-pot) to synthesise a new compound from all the reactants. In green chemistry, MCR is an important method because it is known for its high atomic economy and high structural efficiency of compounds and provides environmental benefits in the preparation of structurally diverse therapeutic compounds [1-6]. Pyrimidine has a distinguished history that extends from its discovery as a significant component of nucleic acids to its current utilization in the chemotherapy of several diseases. It was discovered that the pyrimidine rings are present in vitamins such as thiamin, riboflavin, and folic acid. Various pyrimidine derivatives have been developed over the past two decades as chemotherapeutic agents and have been used for several clinical applications. Benzothiazoles are heterocyclic compounds of importance in biochemistry. There are many fused rings that have been found to have wide applications for pharmacological activities [7]. Several literature reviews revealed a variety of fused ring systems of pyrimidines and their various chemotherapeutic activities, including anticancer [8-10], antioxidant [11], anti-inflammatory, and anti-microbial [12]. Heterocyclic, benzothiazole and pyrimidine constitute two highly active anti-tumor and antimicrobial active drugs. The combination of those two has been expected to have asygnergistic effects upon their biological characteristics. Figure 1 displays two compounds (I and II), which possess good anti-cancer activities [10]. Thiazolopyrimidine derivatives also have systems that possess antitumor activity [13]. Therefore, we focused this work on synthesizing new fused rings of benzothiazole and pyrimidine.

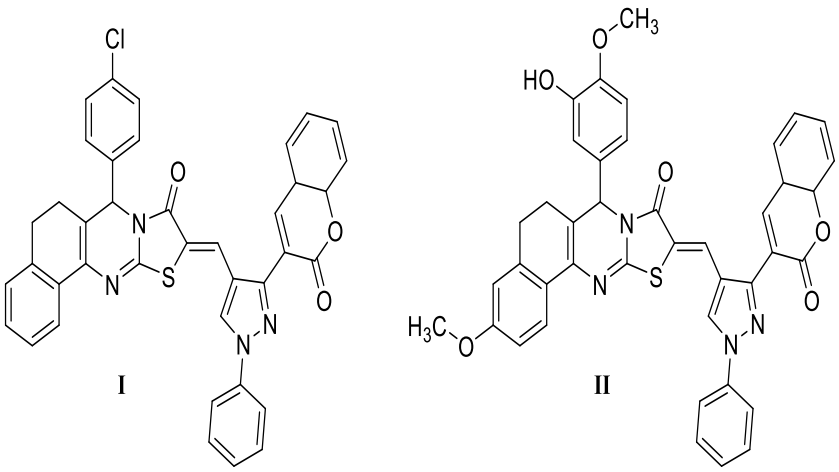


Figure 1: Some synthetic compounds have been reported to be anti-cancer [10].

2. Experimental Part

2.1 Material and Instrumentation

All solvents and chemicals were supplied by the companies Merck, Sigma-Aldrich, and CDH. Thin layer chromatography (TLC) was supplied by the Merck Company, and iodine fumes were used to characterize the spots. A thermal melting point apparatus was used to measure the melting point. A Shimadzu FT-IR Spectrophotometer (FTIR-8400S) has been utilized in order to record FT-IR spectral data at the University of Baghdad / College of Sciences. Nuclear magnetic resonance spectroscopy (400 MHz) was used to record the ^{1}H NMR and ^{13}C NMR data using DMSO-d_6 as a solvent. Chemical shifts were measured in parts per million (ppm) relative to the internal reference tetramethylsilane (TMS) in Iran and the University of Basra in Iraq.

2.1.1 Synthesis of compounds A1-A3 [14,15]

In a one-pot, aldehyde derivatives were added with ethyl acetoacetate, acetylacetone in presence of sodium ethoxide as catalyst in benzene (20 mL) and stirred for 5-10 minutes. 2-Aminobenzothiazole was then added to the mixture and refluxed for 8 hours. This reaction was monitored by the TLC (petroleum ether / ethyl acetate, 4:1). The precipitate was filtered, dried, and recrystallized from ethyl acetate to give the title products **A1-A3.**

Compound A1*: 1-(2-Methyl-4-(2-nitrophenyl)-4H-benzo[4,5]thiazolo[3,2-a]pyrimidin-3-yl) ethan-1-one***:** Yellow color, 61% yield, m.p. 233-235 °C. **FT-IR υ_{max}(cm^{-1}):** 3058 (CH_{aro}), 2974 (CH_{ali}), 1695 (C=O), 1639 (C=C), 1596 (C=N), 1558 and 1496 ($C=C_{aro}$), 1529 and 1336 ($NO_{2asym\ and\ sym}$).

Compound A2*:1-(4-(2,4-Dimethoxyphenyl)-2-methyl-4H-benzo[4,5]thiazolo[3,2-a]pyrimidin -3-yl)ethan-1-one***:** Light yellow color, 78% yield, m.p. 225-227 °C. FT-IR υ_{max}(cm^{-1}): 3041 (CH_{aro}), 2956 (CH_{ali}), 1639 (C=C), 1733 (C=O), 1618 (C=N), 1550 and 1510 ($C=C_{arom}$), 1016 (C-O). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H; 7.66-6.96 (m, Ar-H), 4.81 (s, H, CH), 3.90 - 3.86 (s, 3H, $2OCH_3$), 3.03 (s, 3H, CH_3), 1.62 (s, 3 H,CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C; 153.2-107.2 (C aromatic), 98.6 (N-C pyrimidine), 187.7 (C=O), 166.8 (C=N), 56.4 and 56.2 (2C-O methoxy), 20.8 (CH_3-C=O), 17.5 (CH_3-pyrimidine).

Compound A3*: 1-(4-(2,4-Dichlorophenyl)-2-methyl-4H-benzo[4,5]thiazolo[3,2-a]pyrimidin-3-yl)ethan-1-one*: Beige color, 74% yield, m.p. 215-217°C. FT-IR υ_{max}(cm^{-1}): 3062 (CH_{aro}), 2964 (CH_{ali}), 1639 (C=C), 1610 (C=N), 1697 (C=O), 1583 and 1546 ($C=C_{aro}$), 752(C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H; 8.78-6.90 (m, Ar-H), 4.89 (s, H, CH), 2.37 (s, 3H, CH_3 alkene), 1.33 (s, 3 H, CH_3 ketone). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C; 188.8 (C=O), 167.8 (C=N), 152.2-107.2 (C aromatic), 98.8 (N-C pyrimidine), 21.1 (CH_3-C=O), 18.0 (CH_3-pyrimidine).

2.1.2. Synthesis of compounds A4-A9 [16,17]

To a solution of compounds **A7-A11** (0.01 mmol) in ethanol (15 mL), a few drops of NaOEt (40% in EtOH) were added. The mixture was then stirred for a few minutes before adding the substituted aldehydes (0.01 mmol). After completion of the addition, the mixture was stirred for 24 hours at room temperature. The solvent was then evaporated and the solid material recrystallized from ethanol to afford the desired products **A4-A9**.

Compound A4: *3-(4-Bromophenyl)-1-(2-methyl-4-(2-nitrophenyl)-4H-benzo[4,5]thiazolo [3, 2-a] pyrimidin-3-yl)prop-2-en-1-one***:** Bright red color, 84% yield, m.p. 241-243 °C. FT-IR υ_{max}(cm^{-1}): 3013 (CH_{aro}), 1683(C=O), 2987 (CH_{ali}), 1639 (C=C), 1596 (C=N), 1556 and 1492 ($C=C_{aro}$), 1529 and 1342 ($NO_{2asym\ and\ sym}$). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H; 8.51-6.96 (m, Ar-H), 6.43 (2H, CH=CH), 4.45 (s, 1H, CH), 1.61 (s, 3 H, CH_3)**.** ^{13}C NMR (100 MHz, DMSO-d_6) δ_C; 155.3 (C=N), 171.0 (C=O), 121.8 and 121.4 (C=C), 153.8-123.1 (C_{aro}), 65.4 (N-$C_{pyrimidine}$), 25.8 ($CH_{3pyrimidine}$)**.**

Compound A5*: 3-(4-(Dimethylamino)phenyl)-1-(2-methyl-4-(2-nitrophenyl)-4H-benzo-[4,5]thiazolo [3,2-a]pyrimidin-3-yl)prop-2-en-1-one***:** Maroon color, 86% yield, m.p. 257-259 °C. FT-IR υ_{max}(cm^{-1}): 3004 (CH_{aro}), 1681 (C=O), 2972 (CH_{ali}), 1635 (C=C), overlap C=N with $C=C_{aro}$, 1571 and 1512 ($C=C_{aro}$), 1528 and 1340 ($NO_{2asym\ and\ sym}$).

Compound A6*: 3-(4-Bromophenyl)-1-(4-(2,4-dimethoxyphenyl)-2-methyl-4H benzo[4,5] thiazolo[3,2-a]pyrimidin-3-yl)prop-2-en-1-one*: Orange color, 91% yield, m.p. 250-252 °C. FT-IR υ_{max}(cm^{-1}): 3006 (CH_{aro}), 1639 (C=C), 1683 (C=O), overlap C=N with $C=C_{aro}$, 1560

(C=C_{aro}), 1014 (C-O). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H; 7.78-6.97 (m, Ar-H), 6.68, 6.63 (m, H, CH=CH), 4.45 (s, 1H, CH), 3.90-3.86 (s, 3H, 2OCH_3), 1.61 (s, 3H, CH_3)**.**

Compound A7*: 1-(4-(2,4-Dimethoxyphenyl)-2-methyl-4H-benzo[4,5]thiazolo[3,2-a] pyrimidin-3-yl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one*: Light orange color, 89% yield, m.p. 245-247 ^{o}C. FT-IR υ_{max}(cm^{-1}): 3001 (CH_{aro}), 1681 (C=O), 1639 (C=C), overlap C=N with C=C_{aro}, 1573 (C=C_{aro}), 1014 (C-O).

Compound A8*: 3-(4-Bromophenyl)-1-(4-(2,4-dichlorophenyl)-2-methyl-4H-benzo [4,5] thiazolo[3,2-a]pyrimidin-3-yl)prop-2-en-1-one*: Yellow color, 79% yield, m.p. 260-262 °C. FT-IR υ_{max}(cm^{-1}): 3072 (CH_{aro}), 2974 (CH_{ali}), 1681 (C=O), 1641 (C=C), 1556 (C=C_{aro}), 756 (C-Cl).

Compound A9: 1-(4-(2,4-Dimethoxyphenyl)-2-methyl-4H-benzo[4,5]thiazolo[3,2-a] pyrimidin-3-yl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one; Yellow color, 80% yield, m.p. 269-271 °C. FT-IR υ_{max}(cm^{-1}): 3008 (CH_{aro}), 2983 (CH_{ali}), 1639 (C=C), 1683 (C=O), 1591 (C=N), 1556 (C=C_{aro}), 761(C-Cl).

2.1.3 Synthesis of the compounds A10-A15

A solution of chalcone derivatives A4-A9 (0.01 mmol) in ethanol (20 mL) was treated with a small amount of water until the reaction mixture became clear. After that, urea (0.01 mmol) and Na_2CO_3 (0.01 mmol) were added gradually to the reaction mixture before heating to reflux for 6 hours. After completion of the reaction (monitored by TLC; methanol: benzene, 3:1), the solvent was evaporated and the residue solid material was recrystallized from ethyl acetate to provide the title products A10-A15.

Compound A10*: 6-(4-Bromophenyl)-4-(2-methyl-4-(2-nitrophenyl)-4H-benzo[4,5]thiazolo [3,2-a]pyrimidin-3-yl)pyrimidin-2(1H)-one*: Greyish color, 75% yield, m.p. >300^{o}C. FT-IR υ_{max}(cm^{-1}): 3353 (NH), 3006 (CH_{aro}), 1677(C=O), 1622 and 1610 (2C=N), 1554 (C=$C_{aro.}$), 1520 and 1344 ($NO_{2asym\ and\ sym}$).

Compound A11*: 6-(4-(Dimethylamino)phenyl)-4-(2-methyl-4-(2-nitrophenyl)-4H-benzo[4,5] thiazolo[3,2-a]pyrimidin-3-yl)pyrimidin-2(1H)-one*; Light greyish color, 77% yield, m.p. >300 ^{o}C. FT-IR υ_{max}(cm^{-1}): 3353 (NH), 3002 (CH_{aro}), 2975 (CH_{ali}), 1672 (C=O), 1632 and 1598 (2C=N), overlap C=C_{aro} with NO_{asym}, 1574 (NO_{asym}), and 1352 (NO_{sym}).

Compound A12*: 6-(4-Bromophenyl)-4-(4-(2,4-dimethoxyphenyl)-2-methyl-4H-benzo[4,5] thiazolo [3,2-a]pyrimidin-3-yl)pyrimidin-2(1H)-one*: Light greyish color, 79% yield, m.p. >300^{o}C. FT-IR υ_{max}(cm^{-1}): 3348 (NH), 3070 and 3001 (CH_{aro}), 1679 (C=O), 1600 (C=N), 1628 (C=C), 1575 and 1510 (C=C_{aro}).

Compound A13*: 4-(4-(2,4-Dimethoxyphenyl)-2-methyl-4H-benzo[4,5]thiazolo[3,2-a] pyrimidin-3-yl)-6-(4-(dimethylamino)phenyl)pyrimidin-2(1H)-one*: Orange color, 77% yield, m.p. >300^{o}C. FT-IR υ_{max}(cm^{-1}): 3348 (NH), 3063 and 3007 (CH_{aro}), 1677 (C=O), 1623 (C=C), 1600 (C=N), 1564 and 1510 (C=C_{aro}).

Compound A14*: 6-(4-Bromophenyl)-4-(4-(2,4-dichlorophenyl)-2-methyl-4H-benzo[4,5] thiazolo[3,2-a]pyrimidin-3-yl)pyrimidin-2(1H)-one*: Dark yellow color, 62% yield, m.p. >300^{o}C. FT-IR υ_{max}(cm^{-1}): 3344 (NH), 3060 and 3002 (CH_{aro}), 1622 (C=C), 1676 (C=O), 1600 (C=N), 1581 (C=C_{aro}). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H; 11.20 (s, 1H, NH), 7.66-6.96 (m,

Ar-H), 4.44 (s, 1H, CH), 1.59 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C; 176.8 (C=O), 166.4 and 152.4 (2C=N), 146.8-106.4 (C_{aro}), 102.8 and 102.7 (C=C), 67.7 (N-$C_{pyrimidine}$), 14.0 ($CH_{3pyrimidine}$).

Compound A15*: 4-(4-(2,4-Dichlorophenyl)-2-methyl-4H-benzo[4,5]thiazolo[3,2-a]pyrimidin -3-yl)-6-(4-(dimethylamino)phenyl)pyrimidin-2(1H)-one*; Yellow color, 77% yield, m.p. >300 °C. FT-IR υ_{max}(cm^{-1}): 3342 (NH), 3020 (CH_{aro}), 1672 (C=O), 1622 (C=C), 1600 (C=N), 1580 (C=C_{aro}).

2.1.4. Synthesis of compounds A16-A21 [18,19]

To a mixture of malononitrile (0.01 mmol) and NaOEt (0.01 mmol, 40% in ethanol), chalcone derivatives **A4**-**A9** in ethanol (20 mL) were added before heating to reflux for 7-8 hours. The reaction was monitored by the TLC (methanol:benzene, 3:1). The solvent was then evaporated and the residue solid material was recrystallized from ethyl acetate to provide the desired products **A16**-**A21**.

Compound A16*: 4-(4-Bromophenyl)-2-ethoxy-6-(2-methyl-4-(2-nitrophenyl)-4H-benzo[4,5] thiazolo[3,2-a]pyrimidin-3-yl)nicotinonitrile*: Brown color, 66% yield, m.p. 209-211 °C. FT-IR υ_{max}(cm^{-1}): 3082 (CH_{aro}), 2208-2150 (CN_{nit}), 1650 (C=N), 1637 (C=C), 1581 and 1510 (C=C_{aro}), 1546 and 1377 ($NO_{2asym\ and\ sym}$).

Compound A17*: 4-(4-(Dimethylamino)phenyl)-2-ethoxy-6-(2-methyl-4-(2-nitrophenyl)-4H-benzo[4,5]thiazolo[3,2-a]pyrimidin-3-yl)nicotinonitrile***:** Black color, 64% yield, m.p. 243-245 °C. FT-IR υ_{max}(cm^{-1}): 3099 (CH_{aro}), 2210-2152 (CN_{nit}), 1647 (C=N), 1631 (C=C), 1571 (C=C_{aro}), 1530 and 1377 ($NO_{2asym\ and\ sym}$).

Compound A18*: 4-(4-Bromophenyl)-6-(4-(2,4-dimethoxyphenyl)-2-methyl-4H-benzo[4,5] thiazolo[3,2-a]pyrimidin-3-yl)-2-ethoxynicotinonitrile***;** Dark brown color, 70% yield, m.p. 251-253 °C. FT-IR υ_{max}(cm^{-1}): 3082 (CH_{aro}), 2200 (CN_{nit}), 1645 (C=N), 1632 (C=C), 1575 (C=C_{aro}).

Compound A19*: 6-(4-(2,4-Dimethoxyphenyl)-2-methyl-4H-benzo[4,5]thiazolo[3,2-a]pyrimidin-3-yl)-4-(4-(dimethylamino)phenyl)-2-ethoxynicotinonitrile***:** Light brown color, 64% yield, m.p. 237-239 °C. FT-IR υ_{max}(cm^{-1})**:** 3045 (CH_{aro}), 2316 (CN_{nit}), 1649 (C=N), 1634 (C=C), 1562 (C=C_{aro}). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H; 7.38-6.80 (m, Ar-H), 4.73 (s, 1 H, CH), 4.25 (m, 2H, OCH_2), 3.95-3.84 (s, 6H, OCH_3), 2.99-2.80 (s, 6H, 2N-CH_3), 2.18 (s, 3H, CH_3) 1.63 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C; 166.5-130.2 (C-Ar), 120.7 (CN_{nit}), 116.7 and 116.1 (C=$C_{pyrimidine}$), 111.7 (C=$N_{pyrimidine}$), 82.9 ($C_{pyrimidine}$), 55.0 (O-CH_3), 25.8 (2N-CH_3), 29.0 (C=C-CH_3) and 18.3 (C-CH_3).

Compound A20*: 4-(4-Bromophenyl)-6-(4-(2,4-dichlorophenyl)-2-methyl-4H-benzo[4,5] thiazolo[3,2-a]pyrimidin-3-yl)-2-ethoxynicotinonitrile*: Dark greyish color, 67% yield, m.p. 214-216 °C. FT-IR υ_{max}(cm^{-1}): 3072 (CH_{aro}), 2262 (CN_{nit}), 1647 (C=N), 1573 (C=C_{aro}).

Compound A21*: 6-(4-(2,4-Dichlorophenyl)-2-methyl-4H-benzo[4,5]thiazolo[3,2-a]pyrimidin-3-yl)-4-(4-(dimethylamino)phenyl)-2-ethoxynicotinonitrile***:** Black color, 62% yield, m.p. 220-222 °C. FT-IR υ_{max}(cm^{-1}): 3087 (CH_{aro}), 2204 (CN_{nit}), overlap (C=N), 1635 (C=C), 1541 (C=C_{aro}).

2.2 Cytotoxic Effect of compounds A10 and A19

2.2.1 Cell Line Maintenance

The protocol was applied after the cells in a container were transformed into a confluent monolayer: After aspiration of the growth medium, the cell sheets were washed in phosphate buffer saline (PBS), and trypsin/viresin solution (2-3 mL) was added to the cell. Using a spectrum vibrator, the vessel was completely inverted to a monolayer and left for two minutes at a temperature of 37 ℃ until the cells separated from the vessel in the incubation. Completely fresh Roswell Park Memorial Institute Media 1640 (RPMI) (15-20 mL) was added, and the cells were dispersed from the wedding surface into the growth medium with a pipette. In flasks, the required concentrations were prepared and incubated at a temperature of 37 ^{o}C with 5% carbon dioxide. Cell concentration has been accomplished using a hemometer and by several cells by applying the equation:

Total number of cells/mL = number of cells × dilution factor (or sample volume) × 104

2.2.2 MMT Assay

The cytotoxic impact of different concentration values (12.50, 25, 50, 100, 200 and 400 µg/mL) of the compounds **A10** and **A19** was carried out with the use of the MTT ready-to-use kit:

❖ **Contents of the kit**

- MMT solution 1 mL × 10 tubes.
- Solubilisation solution 50 mL × 2 bottles.

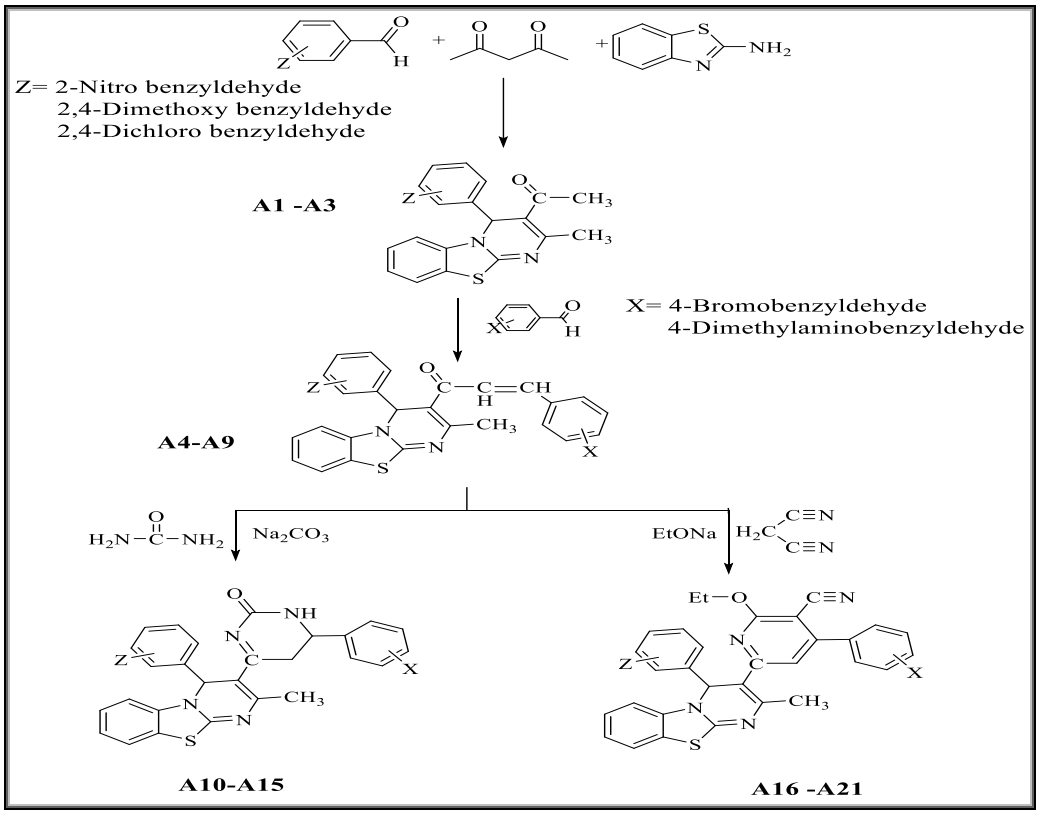
❖ **Assay** [20]

1. Tumor cells (1×10^4-1×10^6 cells/mL) were grown on flat plates (96 µL) with a volume of 200 µL of complete culture medium for each well. The micro-plate was gently shaken and surrounded by sterile Parafilm. At 37°C and CO_2 (5%). The plates were incubated at 37^{o} C with CO_2 (5%) for 24 hours.
2. After removing the medium from compounds **A10** and **A19**, diluted concentrations (12.5, 25, 50, 100, 200, and 400µg/mL) were added to wells.
3. Triplicates were utilized for every one of the concentrations in addition to the controls (cells treated with serum-free medium) under the same conditions.
4. Every well received a 10 L MTT solution before being incubated at CO_2 (5%) for 24 hours.
5. After the incubation period was over, the medium was carefully removed and a solubilisation solution (100µL) was added to every one of the wells and left for 5 minutes.
6. The optical density data measured with the use of an ELISA reader at a wave-length of 575 nm was statistically analysed to assess the concentration of compounds required for the latest decrease in cell viability by 50% for every one of the cell lines.

3. Results and discussion

3.1 Synthesis

All the prepared compounds gave good results in the FT-IR, ^{13}C NMR, and ^{1}H NMR spectroscopy. Scheme 1 shows all the synthesised compounds.



Scheme 1: Synthesis of compounds A1-A21

In the first step, the fused pyrimidine derivatives were prepared using one-pot reactions, where sodium hydroxide helps to form a carbanion, which in turn condenses with the aldehyde (Schmidt reaction) to form an intermediate compound, which in reaction with 2-aminobenzothiazole gives compounds (A1-A3) [14]. The FT-IR spectral data showed stretching bands of the carbonyl group (C=O) in the region of 1733-1695 cm^{-1}, while the NH_2 band had disappeared. Additionally, new bands appear due to C=C at 1639, and C=N in the region 1596-1618 cm^{-1}. The ^1H NMR spectra of A2 and A3 showed many signals due to the aromatic protons between 8.78 and 6.90 ppm, and showed singlet signals of 4.81 and 4.89 ppm belonging to the CH pyrimidine ring. The ^{13}C NMR spectrum of A2 and A3 exhibited signals at 188 and 187 ppm for C=O of ketone, 166 and 167 ppm due to C=N, and 107-153 ppm for C=C aromatic. Chalcones were synthesised by the reaction of compounds A1-A3 with two different aldehydes in the presence of NaOH (40%). The results showed the formation of the desired compounds by FT-IR spectroscopy in the presence of the carbonyl group band in the range of 1681-1683 cm^{-1}. The reason for the lack of frequency is the formation of α,β-unsaturated compounds that contain conjugated systems. The disappearance of the methyl group in the ^1H NMR spectrum and the appearance of signals at 6.65-6.43 ppm due to CH=CH chalcones. As for the ^{13}C NMR spectrum, compound A4 showed a signal at 171.0 ppm that belongs to the carbon carbonyl group, as well as two separate signals at 155.4 and 153.8 owing to C=N and C=C of the pyrimidine rings. In addition to that, the oxopyrimidine derivatives A10-A15 have been synthesized from the reaction of chalcones A4-A9 with urea *via* Michael's addition reaction, and the final structures were identified by FT-IR, ^1H NMR, and ^{13}C NMR spectroscopy. The FT-IR spectral data of these compounds showed new bands at 3353-3348 cm^{-1} due to the NH band. The absorptions in 1677-1672 cm^{-1} are attributed to the carbonyl group in the chalcone. The ^1H NMR spectrum of compound A14 showed a signal at 11.20 ppm that belongs to the NH, which is good evidence for the formation of compound A14. The ^{13}C NMR spectrum displayed a new signal at 176.8 ppm of the carbonyl group for the new

oxopyrimidine ring [21,22]. The nicotinonitrile derivatives A16-A21 were synthesised by Michael's addition from the reaction of the abovementioned chalcones A4-A9 with malononitrile. The products A16-A21 were characterized by FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy. The FT-IR spectra showed the disappearance of the carbonyl group and the appearance of new bands in the range 2316-2150 cm^{-1} belonging to the nitrile group. This spectrum also shows several different signals for the formation of these rings. The ^{1}H NMR spectrum exhibited signals at 4.73, 4.25, 3.95-3.84, 2.99-2.80, 2.18, and 1.63 ppm of this new compound. The ^{13}C NMR spectrum showed the disappearance of carbonyl signals and the appearance of new signals at 111.7 ppm, belonging to the nitrile group [23].

3.2. Cytotoxic effects

One of the aims of this study is to develop a new substance that could be used effectively for treating colon cancer. For this purpose, the cytotoxic effects of compounds A10 and A19 on human colon cancer cells (CaCo-2). Tables 1 and 2 show the cytotoxic effects of samples A10 and A19 on Caco-2 and normal cells after the incubation period (24 hours).

Table 1: Cytotoxicity effects of compound A10 on CaCo-2 and HdFn at 37 ºC cells after the incubation period (24 hours)

Conc. (µg/mL)	Cancer mean ± S.D	Normal Mean ± S.D
400	53.40 ± 3.31	72.65 ± 1.96
200	70.95 ± 8.76	83.54 ± 4.83
100	82.83 ± 1.54	85.65 ± 3.32
50	95.45 ± 0.88	94.17 ± 0.77
25	97.15 ± 1.35	96.18 ± 0.23
12.5	95.87 ± 3.28	94.29 ± 2.98

Table 2: Cytotoxicity effects of compound A19 on CaCo-2 and HdFn at 37 ºC cells after the incubation period (24 hours).

Conc. (µg/mL)	Cancer mean ± S.D	Normal Mean ± S.D
400	42.82 ± 2.77	71.95 ± 0.81
200	45.76 ± 0.72	76.70 ± 2.61
100	63.91 ± 4.88	84.34 ± 2.66
50	71.20 ± 0.43	86.07 ± 1.92
25	83.74 ± 2.34	95.22 ± 0.82
12.5	96.76 ± 1.14	95.95 ± 1.03

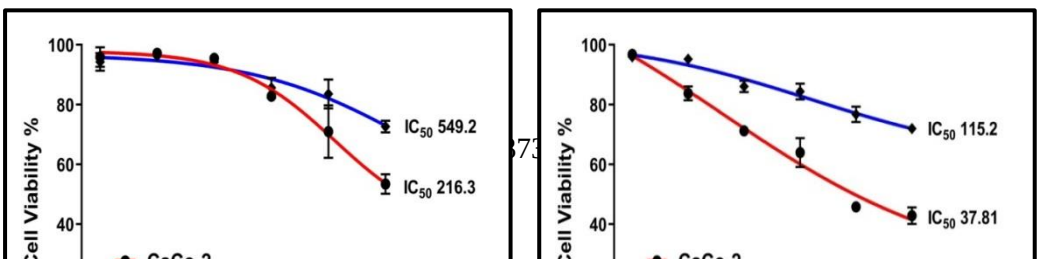


Figure 2 - Cytotoxicity effect of compound **A10** on CaCo-2 and HdFn cells at 37ºC after incubation period (24 hours).

Figure 3 - Cytotoxicity effect of compound **A19** on CaCo-2 and HdFn cells at 37ºC after incubation period (24 hours).

Conclusion

It was verified that compound A10 acted more destructively against Caco-2 colon cancer cells. The test of A10 and A19 was performed to determine the cytotoxic effect of A10 and A19 on the colon cancer cell line (CaCo-2). The MTT assay was designed to calculate the cell viability and inhibition rate of the tumour cell line using various concentrations of compounds. The cytotoxic effect was evaluated at concentrations ranging from 12.5 to 400 µg/mL, as shown in Tables 1 and 2. The results represented a decrease in cell vitality that depends on the dose pattern. Cell viability is maximized by raising the concentration of compounds A10 and A19. The maximum decrease in compound A10 in Caco-2 cell viability (%) was noted at 400 µg/ml (53.40 ± 3.31). Wherever the highest CaCo-2 cell viability was measured, it was at 12.5 µg/mL (95.87 ± 3.28). The synthesis compounds significantly showed the most potent cytotoxic activity, with an IC_{50} value of 549.2µg/mL. However, an IC_{50} of 216.3 µg/mL was obtained from the effect of A10 in the HdFn normal cell line (Figure 2), and the maximum decrease in compound A19 in Caco-2 cell viability (%) was noted at 400 µg/ml (42.82 ± 2.77), while the highest CaCo-2 cell viability at 12.5 µg/mL reached out (96.76 ± 1.14). The synthesis compounds significantly showed the most potent cytotoxic activity, with an IC50 value of 115.2µg/mL. However, an IC_{50} of 37.81 µg/mL was acquired from the effect of A19 in the HdFn normal cell line (Figure 3).

References

[1] S. H. Tan, B. S. Lim, F. S. J. Yong, M. N. Abdullah, H. M. Zuki, S. C. Poh, S. Kan and P. W. Chia, "Synthesis of thiazolo-and benzothiazolo[3,2-a]pyrimidine derivatives using onion peel as natural catalyst", *Songklanakarin Journal of Science and Technology*, vol. 42, no. 1, pp. 57-64, 2020.

[2] I. A. Ibarra, A. Islas-Jácome, and E. González-Zamora, "Synthesis of polyheterocycles via multicomponent reactions", *Organic and Biomolecular Chemistry*, vol. 16, no. 9, pp. 1402-1418, 2018.

[3] M. N. Chen, L. P. Mo, Z. S. Cui, and Z. H. Zhang, "Magnetic nanocatalysts: synthesis and application in multicomponent reactions", *Current Opinion in Green and Sustainable Chemistry*, vol. 15, pp. 27-37, 2019.

[4] S. Zhi, X. Ma, and W. Zhang, "Consecutive multicomponent reactions for the synthesis of complex molecules" *Organic and Biomolecular Chemistry*, vol. 17, no. 33, pp. 7632-7650, 2019.

[5] C. S. Graebin, F. V. Ribeiro, K. R. Rogério, and A. E. Kümmerle, "Multicomponent reactions for the synthesis of bioactive compounds: a review", *Current Organic Synthesis*, vol. 16, no. 6, pp. 855-899, 2019.

[6] S. Garbarino, D. Ravelli, S. Protti, and A. Basso, "Photoinduced multicomponent reactions", *Angewandte Chemie International Edition*, vol. 55, no. 50, pp. 15476-15484, 2016.

[7] A. A. Fadda, S. B. Bondock, A. M. Khalil, and E. H. Tawfik, "Synthesis of Some New 1,2,3, 4-Tetrahydropyrimidine-2-thione and Their Thiazolo[3,2-a]pyrimidine, Thiazino and Benzothiazepine Derivatives", *Journal of Heterocyclic Chemistry*, vol. 50, no. 4, pp. 838-843, 2013.

[8] R. Determann, J. Dreher, K. Baumann, L. Preu, P. G. Jones, F. Totzke, and C. Kunick, "2-Anilino-4-(benzimidazol-2-yl)pyrimidines-A multikinase inhibitor scaffold with antiproliferative activity toward cancer cell lines", *European journal of medicinal chemistry*, vol. 53, pp. 254-263, 2012.

[9] P. M. Lukasik, S. Elabar, F. Lam, H. Shao, X. Liu, A. Y. Abbas, and S. Wang, "Synthesis and biological evaluation of imidazo[4,5-b]pyridine and 4-heteroaryl-pyrimidine derivatives as anti-cancer agents", *European journal of medicinal chemistry*, vol. 57, pp. 311-322, 2012.

[10] R. Gondru, S. R. Peddi, V. Manga, M. Khanapur, R. Gali, N. Sirassu, and R. Bavantula, "One-pot synthesis, biological evaluation and molecular docking studies of fused thiazolo[2,3-b] pyrimidinone-pyrazolylcoumarin hybrids", *Molecular Diversity*, vol. 22, no. 4, pp. 943-956, 2018.

[11] A. A. Hadi, Z. Y. Kadhim, A. N. Seewan, M. G. Munahi, and M. Q. Waheeb, "One-Pot Synthesis of New Pyrazolo[3,4,-d]Pyrimidine Derivatives and Study of their Antioxidant and Anticancer Activities", *Medico-legal Update*, vol. 20, no. 2, pp. 327, 2020.

[12] S. Viveka, G. K. Nagaraja, P. Shama, G. Basavarajaswamy, K. P. Rao, and M. Yanjarappa Sreenivasa, "One pot synthesis of thiazolo[2,3-b]dihydropyrimidinone possessing pyrazole moiety and evaluation of their anti-inflammatory and antimicrobial activities", *Medicinal Chemistry Research*, vol. 27, no. 1, pp. 171-185, 2018.

[13] M. N. Yousif, W. A. El-Sayed, H. A. S. Abbas, H. M. Awad, and N. M. Yousif, "Anticancer activity of new substituted pyrimidines, their thioglycosides and thiazolopyrimidine derivatives", *Journal of applied pharmaceutical science*, vol. 7, no. 11, pp. 021-032, 2017.

[14] C. Fatemeh, D. Fatemeh, and B. Saeed, "A New and Efficient Method for the Synthesis of Pyrimido[2,1-b]benzothiazole Derivatives", *International Journal of Organic Chemistry*, vol. 2, pp. 31-37, 2012.

[15] N. J. Al-Lami "New Imidazo[2,1-b]naphtha[2,1-d][1,3]thiazole Derivatives: Synthesis, Antimicrobial and Antifungal Activity", *Iraqi Journal of Science*, vol. *56*, no. 4C, pp. 3274-3284, 2015.

[16] F. Perdana, Y. Eryanti, and A. Zamri, "Synthesis and toxicity assessments some para-methoxy chalcones derivatives", *Procedia Chemistry*, vol. 16, no. pp. 129-133, 2015.

[17] F. W. Askar, Y. A. Aldheif, N. A. Jinzeel, and O. A. Nief, "Synthesis and Biological Activity Evaluation of New Sulfonamid Derivatives", *Iraqi Journal of Science*, Vol. 58, No. 4B, pp: 2012-2021, 2017.

[18] H. Hegde, R. K. Sinha, S. D. Kulkarni, and N. S. Shetty, "Synthesis, photophysical and DFT studies of naphthyl chalcone and nicotinonitrile derivatives", *Journal of Photochemistry and Photobiology A: Chemistry*, vol. 389, no. 11, pp. 200-222, 2020.

[19] T. A. AL-Sultani Khitam and Naeemah AL-Lami. "Antioxidant Activity of some New synthesised benzo [d] imidazo [2, 1-b] thiazole derivatives." *International Journal of Pharmaceutical Research* vol. 1, pp. 1819-1828, 2020.

[20] R. I. Freshney, 'Culture of animal cells: a manual of basic technique and specialized applications', *John Wiley and Sons*, 2015.

[21] S. G. Khanage , S. A. Raju, P. B. Mohite, and R. B. Pandhare, "Synthesis and pharmacological evaluation of some new pyrimidine derivatives containing 1,2,4-triazole", *Advanced Pharmaceutical Bulletin*, vol. 2, no. 2, pp. 213-222, 2012.

[22] T. S. Ghali, and J. H. Tomma, "Synthesis and characterization of Indazol-3-one and thioxo pyrimidines derivatives from mono and twin chalcones", *Iraqi Journal of Science*, vol. 58, no. 4C, pp. 2265-2277, 2017.

[23] M. J. Joshi, "Studies on Chemical Entities of Therapeutic Interest", Ph.D. thesis, Saurashtra University, INDIA, 2006.