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Synthesis, Characterization of Novel 3-Heteroarylindoles as Inherent to biological activity

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ABSTRACT:

2-(3-(1*H*-Indol-3-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-substituted-5-(substituteddiazenyl)thiazoles and 2-(1*H*-indol-3-yl)-9-substituted-4,7-disubstituted pyrido[3,2-*e*][1,2,4]triazolo[4,3-*a*]pyrimidin-5(7*H*)-ones were synthesized via reaction of hydrazonoyl halides with 3-(1*H*-indol-2-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide and 7-(1*H*-indol-3-yl)-2-thioxo-5-substituted-2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-ones, respectively. Also, hydrazonoyl halides were reacted with *N'*-(1-(1*H*-indol-3-yl)ethylidene)-2-cyanoacetohydrazide to afford 1,3,4-thiadiazole derivatives. Structures of novel synthesis were expounded based on spectral data and elemental analysis besides possibly alternate synthesis routes.

KEYWORDS: thiazoles; pyrazoles; coupling reactions; molecular docking; biological activity.

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INTRODUCTION

The recent research focuses on evaluating the broad spectrum of biological activity that is associated with both natural and synthetic indole derivatives, which assures the feasibility of the synthesis and inherent characteristics of indole derivatives¹⁻⁴. Abundant research on monoindole and bisindole was done intensively and their results inferred that most of the derivatives obtained possess rich biological activities; for illustration, Brassica plants are inherited with indole-3-carbinol, acting as potential anticancer agents^{5,6}.

Thiazoles are widely utilized in the development of drugs for treating allergies⁷, as inhibitors of bacterial DNA gyrase B⁸, sleep disorders⁹, schizophrenia¹⁰, inflammation¹¹, hypertension¹², pain¹⁵, bacterial infections¹⁶, and HIV¹³. It is also used as an inhibitor of bacterial DNA gyrase B⁸ and as fibrinogen receptor antagonists¹⁴. The 1,2,4-triazolopyrimidines attain a phenomenal interest and attraction because of their vital pharmacological characteristics such as macrophage activation, antimalarial, antitumor, antifungal activity, anti-inflammatory and as an antimicrobial agent¹⁷⁻²². Substantial attention also focused on 1,3,4-Thiadiazole derivatives for their broad applications such as antioxidant, antidiabetic, antimicrobial, anticonvulsant activities, CNS depressant, antifungal, molluscicidal, anti-bacterial, anti-hepatitis B viral, analgesic, anti-inflammatory, anti-leishmanial, anti-tuberculosis, diuretic, anti-cancer, anti-tubercular, and anti-hypertensive²³⁻³². Despite the above-mentioned research works, few novel drugs such as 1,3,4-thiadiazole, thiazole, and dihydropyrido[3,2-e][1,2,4]triazolo[4,3-a]pyrimidine derivatives were synthesized with the help of 3-acetylindole as a precursor and evaluated those compounds for their biological activities. Some research has displayed that most of the derivatives of 1,3,4-thiadiazole and thiazole (as represented in Figure 1) possess inherent antitumor activity with admirable IC₅₀ and IG₅₀³³⁻³⁷. Interpreting these evidence, this research explores the synthesis of novel derivatives of 1,3,4-thiadiazoles and thiazoles comprising indole moiety for the assessment against MCF-7 human breast carcinoma cell line to evaluate their anticancer activity.

Anupriya and Starling Sushil, (2013)³⁸ have been reported earlier for more effective drug as antimalarials, acquired the main compound Amodiaquine and substitute Aminoquinoline (A) by phenoxypropanol amines for favourable antimalarials activity. Schiff bases are synthesized from the concentration of an amino group with carbonyl compounds. These compounds and their by-products show a significant function in different biological systems, polymers, dyes and medicinal and pharmaceutical fields were reviewed previously by Meenachi and Chitra, 2013³⁹. Chemical structures of the newly synthesised 3-Hetero arylindoles were evaluated based on elemental analysis, spectral data, and other synthetic routes whenever possible. These results showed fifteen of the new

compounds have been assessed for their antitumor activity against the MCF-7 human breast carcinoma cell line. These result findings designated that several of the tested compounds displayed reasonable to increased anticancer activity (Abdelhamid, et al., 2016)⁴⁰. Bisindole have been intensively investigated and the consequences exposed that most of them have biological activities, indole-3-carbinol, found in *Brassica* plants, is a potential cancer protective agent⁴¹. Thiazoles can be established in drug development for the treatment of allergies⁴², hypertension⁴³, inflammation⁴⁴,

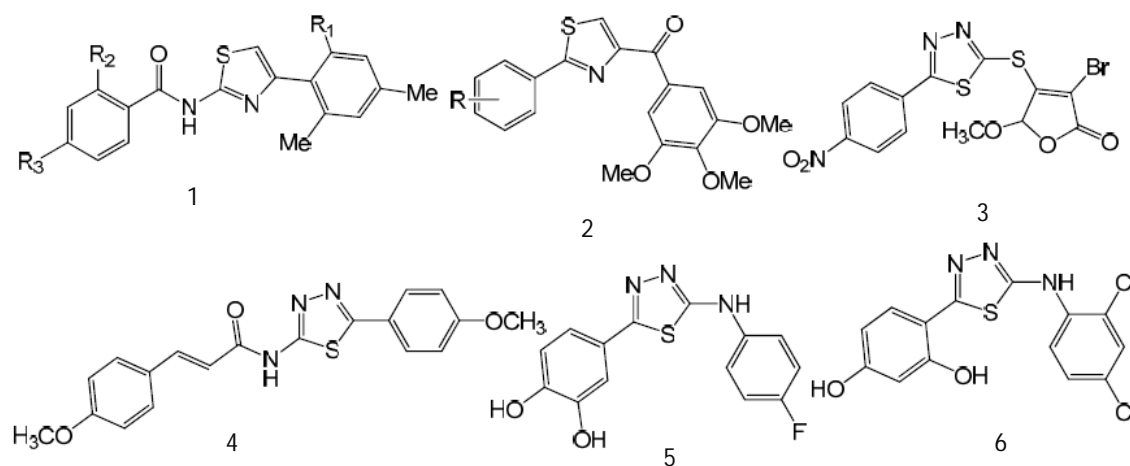
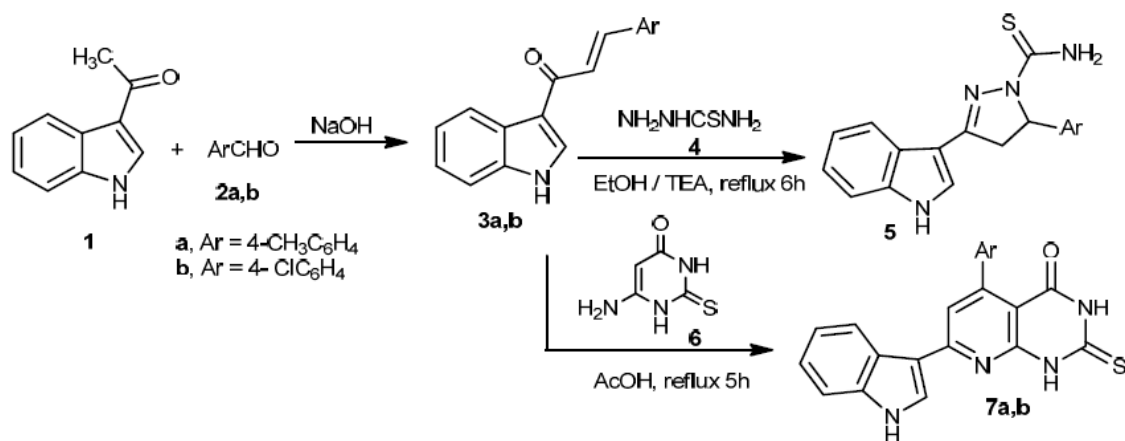


Figure 1. Lead compounds among thiazole and thiazadiazole derivatives with anticancer activity³³⁻³⁷

Synthesis and Methodology

Chemistry

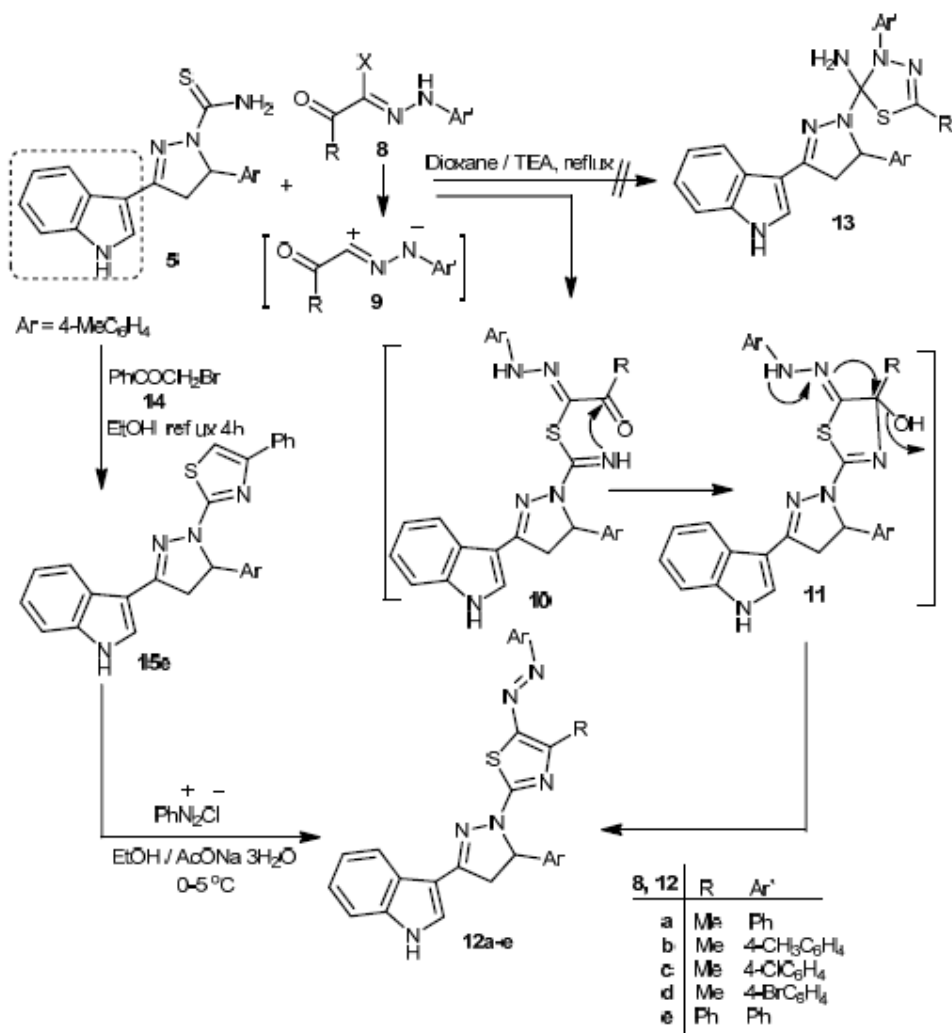
From the reaction of 3-acetylindole (**1**) with 4-chlorobenzaldehyde (**2b**) and *p*-tolualdehyde (**2a**) respectively (Scheme 1), 3-(4-chlorophenyl)-1-(1*H*-indol-3-yl)prop-2-en-1-one (**3b**)⁴⁵ and 1-(1*H*-Indol-3-yl)-3-(*p*-tolyl)prop-2-en-1-one (**3a**)⁴⁶ were prepared as stated in the above literature.



Scheme 1. Synthesis of pyrazole-1-carbothioamide **5** and pyrido[2,3-*d*]pyrimidinthione derivatives **7a,b**.

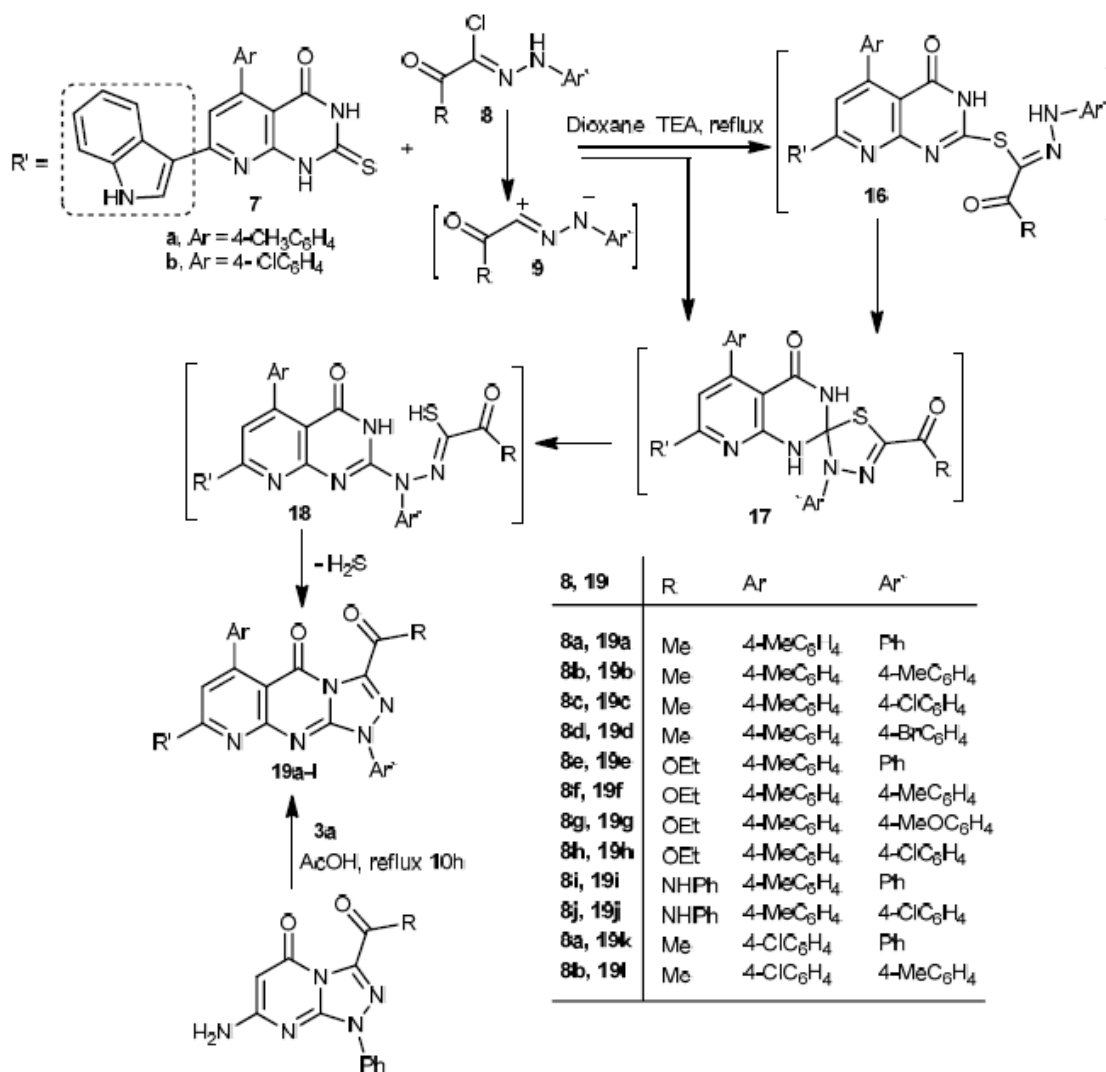
Treatment of 3-aryl-1-(1*H*-indol-3-yl)prop-2-en-1-ones **3a,b** with thiosemicarbazide (**4**) afforded 3-(1*H*-indol-2-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazole-1-carbthioamide (**5**). Similar treatment with 6-amino-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (**6**) generated 5-aryl-7-(1*H*-indol-3-yl)-2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-ones **7a,b**, respectively (Scheme 1). Structures **5** and **7** were expounded by chemical transformation, elemental analysis and spectral data.

Compound **5** was reacted with the appropriate keto-hydrazoneyl halides **8a-e** in dioxane containing a catalytic amount of TEA, to give 2-(3-(1*H*-indol-3-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-5-(aryldiazenyl)-4-substituted thiazoles **12a-e**. Coupling of 2-(3-(1*H*-indol-3-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazole (**15e**) [prepared by the reaction of **5** with phenacyl bromide (**14**)] with benzene diazonium chloride in ethanolic sodium acetate solution at 0°C synthesized a product which is identical to **12e** in all aspects (mp, mixed mp, and spectra). In spite of these results, the mechanism outlined in Scheme 2 seems to be the most plausible pathway for the formation of **12a** from the reaction of **5** with **8a**. The reaction involves an initial formation of thiohydrazone **10**, which undergoes cyclization as soon as it forms an intermediate **11**. The latter loses one molecule of water to give the final product **12a**. Alternatively, 1,3-dipolar cycloaddition of nitrilimine **9a** [prepared in situ from **8a** with triethylamine] to the C=S double bond of **5** could also lead to **10**. The formation of **11** and **12** are similar to that of previous reported reactions of hydrazoneyl chloride with 1-phenyl-1,4-dihydro-1,2,4-triazole-5-thione⁴⁷ and 5-phenyl-1,3,4-thiadiazole-2(3*H*)-thione⁴⁸. Another possible product, 1-(5-(3-(1*H*-indol-3-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-5-amino-4-phenyl-4,5-dihydro-1,3,4-thiadiazol-2-yl)ethan-1-one (**13**), was ruled out by elemental analysis and spectral data analysis. Analogously, the treatment of appropriate **5** with **8b-e** resulted in 2-(3-(1*H*-indol-3-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-substituted-5-(substituted-diazenyl)thiazoles **12b-e**, respectively, with good yield (Scheme 2).



Scheme 2. Synthesis of arylazothiazole derivatives 12a-e.

Treatment of the appropriate 5-aryl-7-(1H-indol-3-yl)-2-thioxo-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-ones **7a** or **7b** with the appropriate hydrazonoyl halides **8a-j** in dioxane containing TEA under reflux produced 2-(1H-indol-3-yl)-9-substituted-4,7-disubstituted pyrido[3,2-e][1,2,4]triazolo[4,3-a]pyrimidin-5(7H)-ones **19a-1**, respectively (Scheme 3). Structure, **19** was expounded by elemental analysis, spectral data analysis, and alternative synthesis routes. Thus, the treatment of 7-amino-3-substituted-1-phenyl-[1,2,4]triazolo[4,3-a]pyrimidin-5(1H)-ones **20a,e,i**⁴⁹ with chalcone **3a** in boiling acetic acid produced the products that were identical in all respects (mp., mixed mp., and spectra) with the corresponding **19a,e,i**.

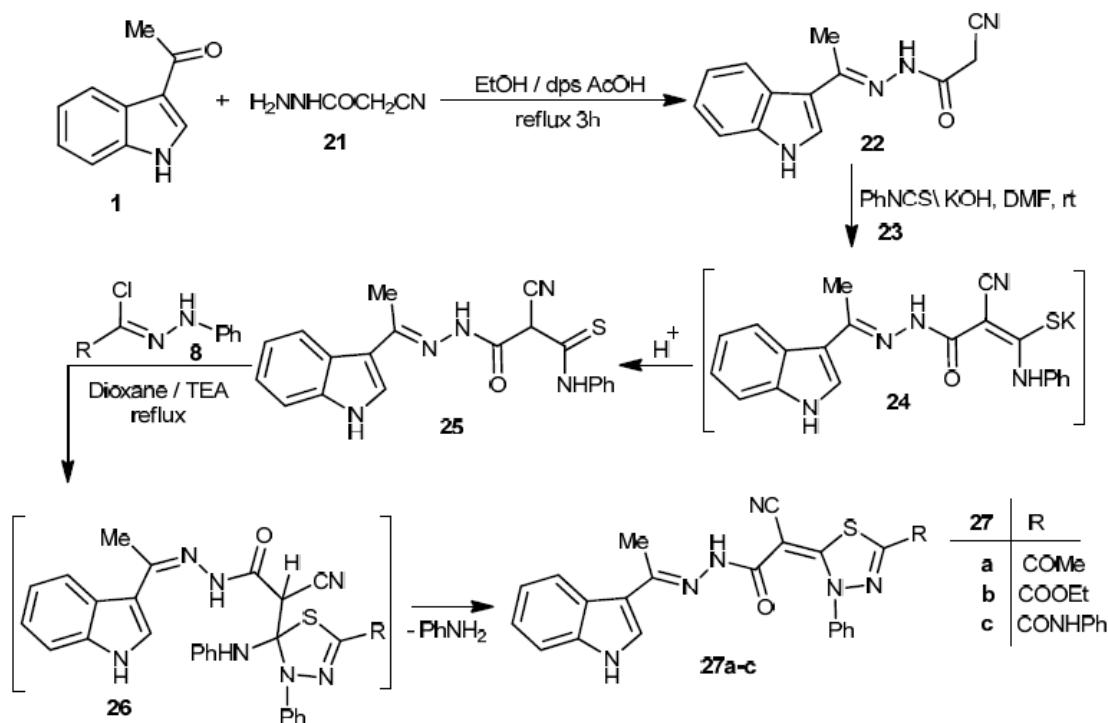


Scheme 3. Synthesis of triazolopyridopyrimidinones 19a-l.

The mechanism in Scheme 3 outline seems to be the most plausible pathway for the formation of **19** from the reaction of thione **7** with **8** via two pathways. In the first pathway, 1,3-addition of the thiol tautomer **7** to the nitrilimine **9** would give the thiohydrazone ester **16** which could undergo nucleophilic cyclization in order to yield spiro compounds **17**. Ring opening to produce **18** is followed by cyclization with loss of hydrogen sulphide would then yield **19**. In the second pathway, an initial 1,3-cycloaddition of nitrilimine **9** to the C=S double bond of **7** would give **17** directly (Scheme 3). Attempts that made for the isolation of thiohydrazone ester **16**, spiro intermediate **17** and thiohydrazone **18** did not succeed, even under mild conditions, as they readily undergo in situ cyclization, which is then followed by the elimination of hydrogen sulphide to give the final product **19**. This structural assignment is also consistent with literature reports, which

indicated the reaction of hydrazonoyl halides with 2-thioxo-pyrimidin-4-one yielded the corresponding 1,2,4-triazolo [4,3-a] pyrimidin-5-one derivatives⁵⁰.

Finally, 2-cyanoacetohydrazide (**21**) was reacted with 3-acetylindole (**1**) in boiling ethanol containing a catalytic amount of acetic acid to afford N'-(1-(1H-indol-3-yl) ethylidene)-2-cyanoacetohydrazide (**22**) with good yield (Scheme 4). Structure **22** was elucidated by elemental analysis, spectral data analysis, and chemical transformation. Treatment of **22** with phenyl isothiocyanate (**23**) in DMF with potassium hydroxide at room temperature followed by the acid work-up has yielded the thioanilide **25**. Refluxing thioanilide **25** with the appropriate hydrazonoyl chlorides **8a,e,i** in ethanolic triethylamine, gave the respective 1,3,4-thiadiazoles **27a-c** (Scheme 4). Thiadiazole **27a-c** was elucidated by elemental analysis and spectral data analysis.



Scheme 4. Synthesis of thiadiazoles **27a-c**.

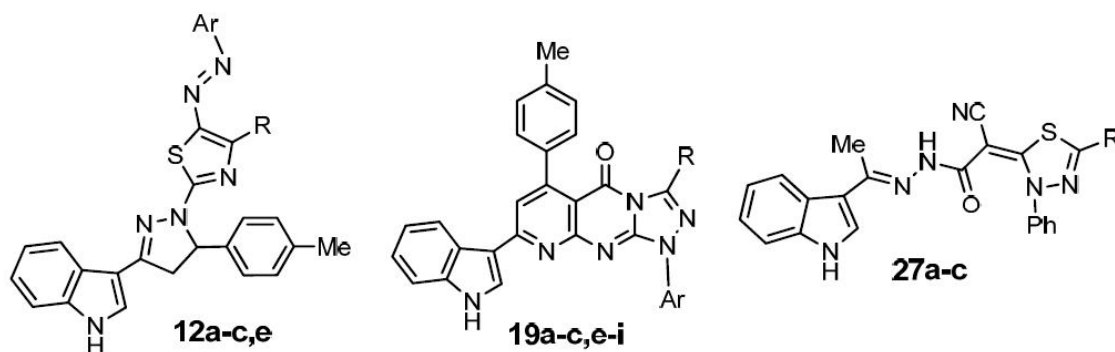
Biological Screening (Cytotoxic Activity)

The *in vitro* growth inhibitory rates (%) and inhibitory growth activity (as measured by IC_{50}) of the newly synthesized compounds were determined against the MCF-7 human breast carcinoma cell line in comparison with the well-known anticancer drug named doxorubicin as the standard, using the MTT viability assay. The data generated from this experiment was used to plot a dose-response curve. From the plot, the concentration (μM) of test compounds required to kill 50% of

cell population (IC_{50}) was determined. Cytotoxicity was expressed as the mean IC_{50} of three independent experiments. The difference between inhibitory activities of all compounds at different concentrations was statistically significant $p < 0.001$.

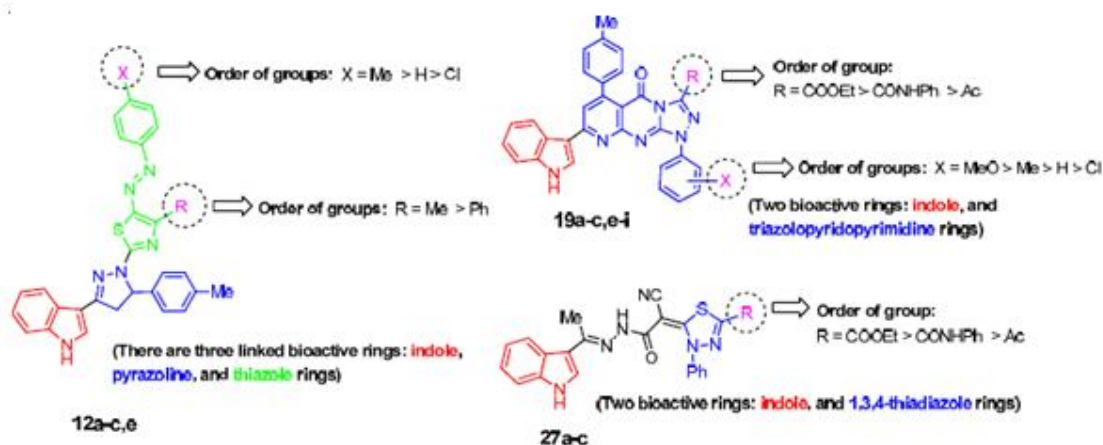
The results revealed that the tested compounds exhibit high variation in the inhibitory growth rates and activities against the tested tumour cell lines in a concentration-dependent manner compared to the reference drug as shown in Table 1 and Scheme 5.

Table 1. The antitumor activities of the tested compounds were compared with reference to doxorubicin using MTT assay on the MCF-7 breast cancer cell line.



Compound No.	R	Ar	IC_{50} (μ M)
12a	CH ₃	Ph	4.92
12b	CH ₃	4-MeC ₆ H ₄	0.95
12c	CH ₃	4-ClC ₆ H ₄	14.52
12e	Ph	Ph	19.44

Compound No.	R	Ar	IC ₅₀ (μM)
19a	CH ₃ CO	Ph	6.88
19b	CH ₃ CO	4-MeC ₆ H ₄	4.68
19c	CH ₃ CO	4-ClC ₆ H ₄	69.85
19e	CH ₃ CH ₂ OCO	Ph	4.83
19f	CH ₃ CH ₂ OCO	4-MeC ₆ H ₄	5.49
19g	CH ₃ CH ₂ OCO	4-MeOC ₆ H ₄	3.05
19h	CH ₃ CH ₂ OCO	4-ClC ₆ H ₄	18.61
19i	PhNHCO	Ph	6.07
27a	CH ₃ CO	Ph	2.04
27b	CH ₃ CH ₂ OCO	Ph	1.01
27c	PhNHCO	Ph	1.27
Doxorubicin	-	-	0.75



Scheme 5. Activities of tested compounds against the MCF-7 breast cancer cell line.

The descending order of the activity of newly synthesized compounds was as follows:

12b > 27b > 27c > 27a > 19g > 19b > 19e > 12a > 19f > 19i > 19a > 12c > 19h > 12e > 19c.

Examination of the Compound Activities Leads to the Following Conclusions

- The various activities of the synthesized compounds depend on the skeletal structure and the electronic configuration of molecules.
- Based on our limited study, the 1, 3, 4-thiadiazole ring as in **27** has *in vitro* inhibitory activity greater than the 1,3-thiazole ring in **12** and triazolopyridopyrimidine ring in **19**.

For the 1,3-Thiazole Ring 12a-c,e

- The *in vitro* inhibitory activity of the 4-methylthiazole is greater than 4-phenylthiazole (**12a** > **12e**). This might be due to the positive inductive effect (+I effect) of the methyl group (increase activity) or the steric hindrance caused by phenyl group (decreased activity).

- The introduction of an electron-donating group (methyl) at C4 position of the phenyl group in the 1,3-thiazole ring enhances the antitumor activity. In contrast, introduction of an electron-acceptor group (chlorine) decreases the antitumor activity (**12b**>**12a**>**12c**).

For Triazolopyridopyrimidines 19a-c,e,i

- For substituent at position 3: the ester group (CO₂Et) gives higher activity than the amide group (CONHPh) or the acetyl group (Ac) (**19e**>**19i**>**19a**).
- Generally, on fixing the substituents at position 3, the electron-donating group (methyl or methoxy) at C4 of the phenyl ring enhances the antitumor activity while the electron-withdrawing group (chlorine) decreases the antitumor activity (**19b**>**19a**>**19c** and **19g**>**19f**>**19e**>**19h**).

For 1,3,4-Thiadiazoles 27a-c

- The *in vitro* inhibitory activities of the compounds with substituents at position 5 are in the order of: COOEt>CONHPh> CH₃CO (**27b**>**27c**>**27a**).

EXPERIMENTAL

Chemistry

All melting points were measured using an Electro thermal digital melting point apparatus. The IR spectra were recorded on potassium bromide discs PC infrared spectrophotometer. The NMR Spectra were recorded at 270 MHz using an NMR spectrometer operating at 300 MHz (¹H-NMR) and was run in deuterated dimethylsulfoxide (DMSO-d₆). Chemical shifts were related to that of the solvent. ¹³C-NMR spectra were also recorded at 75 MHz. Mass spectra were recorded at 70 eV. Elemental analyses and the biological evaluation of the products were performed. All reactions were followed by TLC. Hydrazonoyl halides were prepared as reported in the literature⁵¹⁻⁵⁴.

Synthesis of 3-(1H-Indol-3-yl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5)

To a mixture of 1-(1H-indol-3-yl)-3-(p-tolyl)prop-2-en-1-one (**3a**) (2.61 g, 10 mmol) and thiosemicarbazide (0.92 g, 10 mmol) in EtOH (20 mL), a catalytic amount of triethylamine (1 mL) was added, then heated under reflux for 6 hours. The residual solid was collected, washed with EtOH and recrystallized from acetic acid to give a pure **5** as a white solid (74%); mp 179–181 °C; IR (KBr): ν 3426, 3212, 3175 (NH₂ and NH), 1569 (C=N) cm⁻¹; ¹H-NMR (DMSO-d₆): δ 2.35 (s, 3H, CH₃), 3.17 (dd, 1H, H_A, J = 17.2, 6.3 Hz), 3.46 (dd, 1H, H_B, J = 17.2, 12.1 Hz), 5.87 (dd, 1H, H_X, J =

12.2, 6.3 Hz), 7.02–8.34(m, 8H, Ar-H), 8.72 (s, 1H, Indole-H₂), 11.54 (br s, 2H, NH₂), 12.10 (br s, 1H, NH); MS *m/z* (%): 334(M⁺, 11), 228 (43), 196 (48), 109 (100), 79 (29), 52 (27). Anal. Calcd. for C₁₉H₁₈N₄S (334.44): C, 68.23; H, 5.42; N, 16.75. Found C, 68.18; H, 5.35; N, 16.59.

Synthesis of Thiones (7a,b)

A mixture of chalcones **3a,b** (10 mmol) and 6-amino-2-thioxo-2,3,4-trihydro-1*H*-pyrimidin-4-one (**6**) (1.43 g, 10 mmol) in glacial acetic acid (30 mL) was heated under reflux for 5 hours. After cooling, the reaction mixture was poured into an ice/concHCl mixture and the formed solid was collected and recrystallized from DMF to give thiones **7a,b**, respectively.

7-(1*H*-Indol-3-yl)-2-thioxo-5-(*p*-tolyl)-2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-one (**7a**). Yellow crystals, 72%, mp 265–267 °C; IR (KBr): ν 3442, 3392, 3271 (3NH), 1675 (C=O), 1595 (C=N) cm⁻¹; ¹H-NMR (DMSO-*d*₆): δ 2.44 (s, 3H, CH₃), 4.69 (br s, 1H, NH), 7.15–8.28 (m, 9H, Ar-H and pyridine-H), 8.69 (s, 1H, indole-H₂), 11.46 (br s, 1H, NH), 11.88 (br s, 1H, NH); MS, *m/z* (%) 384 (M⁺, 23), 249 (68), 192 (100), 119 (26), 73 (83). Anal. Calcd. for C₂₂H₁₆N₄OS (384.45): C, 68.73; H, 4.19; N, 14.57. Found: C, 68.59; H, 4.06; N, 14.43.

5-(4-Chlorophenyl)-7-(1*H*-indol-3-yl)-2-thioxo-2,3-dihydropyridol[2,3-*d*]pyrimidin-4(1*H*)-one (**7b**). Yellow crystals, 76%, mp 276–278 °C; IR (KBr): ν 3463, 3368, 3163 (3NH), 1678 (C=O), 1595 (C=N) cm⁻¹; ¹H-NMR (DMSO-*d*₆): δ 2.44 (s, 3H, CH₃), 4.70 (br s, 1H, NH), 7.10–8.32 (m, 9H, Ar-H and pyridine-H), 8.72 (s, 1H, indole-H₂), 11.48 (br s, 1H, NH), 12.04 (br s, 1H, NH); MS, *m/z* (%) 404 (M⁺, 3), 278 (100), 151 (66), 105 (18), 57 (23). Anal. Calcd. for C₂₁H₁₃ClN₄OS (404.87): C, 62.30; H, 3.24; N, 13.84. Found: C, 62.18; H, 3.20; N, 13.65.

Synthesis of 2-(3-(1*H*-Indol-3-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-substituted-5-(aryl diazenyl)thiazole (**12a–e**)

A mixture of 3-(1*H*-indol-3-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (**5**) (0.334 g, 1 mmol) and the appropriate hydrazonoyl halides **8a–e** (1 mmol) in dioxane (20 mL) containing TEA (0.5 mL) was refluxed for 4 hours (monitored by TLC), then allowed to cool. Later the solid formed was collected, washed with EtOH, dried, and recrystallized from DMF to give **12a–e**.

2-(3-(1*H*-Indol-3-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-methyl-5-(phenyldiazenyl)thiazole (**12a**). Red solid, (78% yield); mp 210–212 °C; IR (KBr): ν 3409 (NH), 1642, 1593 (C=N)

cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 2.35 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 3.24 (dd, 1H, H_A , $J = 17.2, 6.3$ Hz), 3.40 (dd, 1H, H_B , $J = 17.2, 12.1$ Hz), 5.88 (dd, 1H, H_X , $J = 12.2, 6.3$ Hz), 7.17–8.34 (m, 13H, Ar-H), 8.72 (s, 1H, indole- H_2), 12.10 (br s, 1H, NH); $^{13}\text{C-NMR}$ (DMSO- d_6): δ 12.2, 21.0, 36.3, 68.0, 109.5, 111.1, 114.8, 117.1, 119.8, 121.7, 128.8, 130.2, 130.5, 131.4, 133.2, 138.2, 136.1, 147.8, 150.1, 154.0, 161.2; MS, m/z (%) 476 (M^+ , 61), 349 (19), 249 (44), 152 (50), 29 (100). Anal. Calcd. for $\text{C}_{28}\text{H}_{24}\text{N}_6\text{S}$ (476.60): C, 70.56; H, 5.08; N, 17.63. Found: C, 70.47; H, 5.01; N, 17.53.

2-(3-(1*H*-Indol-3-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-methyl-5-(*p*-tolyl-diazenyl)thiazole (**12b**). Red solid, (72% yield); mp 193–195 °C; IR (KBr): ν 3403 (NH), 1642, 1590 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 2.18 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 3.05 (dd, 1H, H_A , $J = 17.2, 6.3$ Hz), 3.57 (dd, 1H, H_B , $J = 17.2, 12.1$ Hz), 5.84 (dd, 1H, H_X , $J = 12.2, 6.3$ Hz), 7.12–8.34 (m, 12H, Ar-H), 8.71 (s, 1H, indole- H_2), 12.09 (br s, 1H, NH); MS, m/z (%) 490 (M^+ , 2), 368 (100), 255 (26), 147 (40), 105 (37), 91(41), 55 (40). Anal. Calcd. for $\text{C}_{29}\text{H}_{26}\text{N}_6\text{S}$ (490.62): C, 70.99; H, 5.34; N, 17.13. Found: C, 70.76; H, 5.22; N, 17.05.

2-(3-(1*H*-Indol-3-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-5-((4-chlorophenyl)diazenyl)-4-methyl-thiazole(**12c**). Red solid, (75% yield); mp 206–208 °C; IR (KBr): ν 3403 (NH), 1642, 1590 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): 2.35 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 3.28 (dd, 1H, H_A , $J = 17.2, 6.3$ Hz), 3.57 (dd, 1H, H_B , $J = 17.2, 12.1$ Hz), 5.80 (dd, 1H, H_X , $J = 12.2, 6.3$ Hz), 7.17–8.34 (m, 12H, Ar-H), 8.72 (s, 1H, indole- H_2), 12.09 (br s, 1H, NH); MS, m/z (%) 511 (M^+ , 34), 452 (69), 262 (86), 189 (100), 136 (37), 95 (48), 43 (50). Anal. Calcd. for $\text{C}_{28}\text{H}_{23}\text{ClN}_6\text{S}$ (511.04): C, 65.81; H, 4.54; N, 16.44. Found: C, 65.65; H, 4.37; N, 16.30.

2-(3-(1*H*-Indol-3-yl)-5-(*p*-tosyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-5-((4-bromophenyl) diazenyl)-4-methyl-thiazole(**12d**). Red solid, (78% yield); mp 186–188 °C; IR (KBr): ν 3409 (NH), 1641, 1586 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 2.35 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 3.23 (dd, 1H, H_A , $J = 17.2, 6.3$ Hz), 3.56 (dd, 1H, H_B , $J = 17.2, 12.1$ Hz), 5.78 (dd, 1H, H_X , $J = 12.2, 6.3$ Hz), 7.17–8.34 (m, 12H, Ar-H), 8.71 (s, 1H, indole- H_2), 12.09 (br s, 1H, NH); MS, m/z (%) 556 (M^+ , 12), 370 (37), 248 (60), 235 (100), 91 (48), 55 (36). Anal. Calcd. for $\text{C}_{28}\text{H}_{23}\text{BrN}_6\text{S}$ (555.49): C, 60.54; H, 4.17; N, 15.13. Found: C, 60.48; H, 4.11; N, 15.06.

2-(3-(1*H*-Indol-3-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenyl-5-(phenyldiazenyl)thiazole (**12e**). Red solid, (72% yield); mp 232–234 °C; IR (KBr): ν 3404 (NH), 1640, 1591 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 2.35 (s, 3H, CH_3), 3.25 (dd, 1H, H_A , $J = 17.2, 6.3$ Hz), 3.48 (dd, 1H,

H_B , $J = 17.2, 12.1$ Hz), 5.84 (dd, 1H, H_X , $J = 12.2, 6.3$ Hz), 7.17–8.34 (m, 18H, Ar-H), 8.72 (s, 1H, indole-H₂), 12.08 (br s, 1H, NH); ¹³C-NMR (DMSO-d₆): δ 21.0, 35.8, 68.0, 106.0, 109.5, 110.9, 117.3, 119.8, 121.4, 121.2, 125.4, 128.1, 128.4, 129.5, 130.1, 130.4, 131.4, 133.0, 133.4, 135.1, 136.0, 136.4, 147.8, 150.6, 154.6, 168.1; MS, m/z (%) 538 (M^+ , 9), 451 (4), 432 (43), 326 (62), 225 (100), 77 (64). Anal. Calcd for C₃₃H₂₆N₆S (538.66): C, 73.58; H, 4.87; N, 15.60. Found: C, 73.49; H, 4.79; N, 15.47.

Alternate Synthesis of **12e**

Synthesis of 2-(3-(1*H*-indol-3-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazole (**15e**). A mixture of 3-(1*H*-indol-3-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (**5**) (0.668 g, 2 mmol) and phenacyl bromide (**14**) (0.398 g, 2 mmol) in absolute EtOH (30 mL) was refluxed for 4 hours. The product was started to separate out during the course of reaction. The crystalline solid was filtered, washed with water, dried, and recrystallized from EtOH to give pure thiazole **15e** as yellow crystals in 77% yield; mp 177–179 °C; IR (KBr) ν 3389 (CH), 1616 (C=N) cm⁻¹; ¹H-NMR (300 MHz, DMSO-d₆): δ 2.34 (s, 3H, CH₃), 3.17 (dd, 1H, H_A , $J = 17.6, 6.1$ Hz), 4.19 (dd, 1H, H_B , $J = 17.6, 12.2$ Hz), 5.80 (dd, 1H, H_X , $J = 12.4, 6.1$ Hz), 6.85 (s, 1H, thiazole-H₅), 7.25–8.32 (m, 13H, Ar-H), 8.72 (s, 1H, indole-H₂), 12.10 (br s, 1H, NH); MS, m/z (%) 434 (M^+ , 2), 324 (37), 225 (100), 183 (68), 157 (74), 72 (58). Anal. Calcd. For C₂₇H₂₂N₄S (434.56): C, 74.63; H, 5.10; N, 12.89. Found: C, 74.59; H, 5.07; N, 12.69.

Coupling of Thiazole **15e** with Benzenediazonium Chloride

To a solution of **15e** (0.434 g, 1 mmol) in EtOH (20 mL), sodium acetate trihydrate (0.138 g, 1 mmol) was added and the mixture was cooled to 0–5 °C in an ice bath. To the resulting cold solution, a proportion of benzenediazonium chloride was added (prepared by diazotizing aniline (1 mmol) and dissolved in hydrochloric acid (6 M, 1 mL) with a solution of sodium nitrite (0.07 g, 1 mmol) in water (2 mL)). After the complete addition of diazonium salt, the reaction mixture was stirred further for 30 minutes in an ice bath. The separated solid was filtered off, washed with water, and finally recrystallized from DMF to give a product that proved identical in all respects (mp, mixed mp and IR spectra) with compound **12e** obtained from reaction of **5** with **8e** in 70% yield.

General Procedure for the Reaction of Hydrazonoyl Halides **8** with Thiones **7a,b**

To a solution of thione **7a** or **7b** (1 mmol), appropriate hydrazonoyl halides **8** (1 mmol) in dioxane (20 mL) were added TEA (0.14 mL, 1 mmol). The reaction mixture was refluxed until all of the starting materials had disappeared (8–12 hours, monitored by TLC). The solvent was

evaporated and the residue was triturated with MeOH. The solid formed was collected and recrystallized from the appropriate solvent to give products **19a–l**. The products **19a–l** together with their physical constants are listed below.

9-Acetyl-2-(1*H*-indol-3-yl)-7-phenyl-4-(*p*-tolyl)pyrido[3,2-*e*][1,2,4]triazolo[4,3-*a*]pyrimidin-5 (7*H*)-one (**19a**). Yellow solid, (80% yield), mp 253–255 °C; IR (KBr): ν 3435 (NH), 1706, 1633 (2C=O), 1590 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 2.35 (s, 3H, CH₃), 2.49 (s, 3H, CH₃), 7.30–8.38 (m, 14H, Ar-H and pyridine-H), 8.72 (s, 1H, indole-H₂), 12.02 (br s, 1H, NH); $^{13}\text{C-NMR}$ (DMSO- d_6) d : 21.0, 25.5, 109.4, 113.6, 115.8, 119.0, 120.9, 121.0, 123.9, 126.2, 127.1, 129.2, 129.6, 131.4, 131.8, 133.8, 136.4, 140.9, 146.2, 148.4, 161.0, 163.9, 167.1, 180.6; MS, m/z (%) 510 (M⁺, 36), 407 (23), 334 (22), 233 (27), 105 (100), 77 (22). Anal. Calcd. for C₃₁H₂₂N₆O₂ (510.55): C, 72.93; H, 4.34; N, 16.46. Found: C, 72.76; H, 4.32; N, 16.28.

9-Acetyl-2-(1*H*-indol-3-yl)-4,7-di-*p*-tolylpyrido[3,2-*e*][1,2,4]triazolo[4,3-*a*]pyrimidin-5 (7*H*)-one (**19b**). Yellow solid, (76% yield), mp 242–244 °C; IR (KBr): ν 3421 (NH), 1707, 1642 (2C=O), 1589 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 2.23 (s, 3H, CH₃), 2.34 (s, 3H, CH₃), 2.48 (s, 3H, CH₃), 7.07–8.28 (m, 13H, Ar-H and pyridine-H), 8.70 (s, 1H, indole-H₂), 12.03 (br s, 1H, NH); MS, m/z (%) 524 (M⁺, 22), 509 (100), 381 (32), 231 (96), 173 (79), 55 (63). Anal. Calcd. for C₃₂H₂₄N₆O₂ (524.57): C, 73.27; H, 4.61; N, 16.02. Found: C, 73.12; H, 4.48; N, 15.93.

9-Acetyl-7-(4-chlorophenyl)-2-(1*H*-indol-3-yl)-4-(*p*-tolyl)pyrido[3,2-*e*][1,2,4]triazolo[4,3-*a*]pyrimidin-5 (7*H*)-one (**19c**). Yellow solid, (83% yield), mp 264–265 °C; IR (KBr): ν 3386 (NH), 1710, 1644 (2C=O), 1589 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 2.35 (s, 3H, CH₃), 2.44 (s, 3H, CH₃), 7.09–8.34 (m, 13H, Ar-H and pyridine-H), 8.71 (s, 1H, indole-H₂), 12.09 (br s, 1H, NH); MS, m/z (%) 545 (M⁺, 3), 490 (24), 258 (30), 152 (47), 29 (100). Anal. Calcd. for C₃₁H₂₁ClN₆O₂ (544.99): C, 68.32; H, 3.88; N, 15.42. Found: C, 68.20; H, 3.69; N, 15.31.

9-Acetyl-7-(4-bromophenyl)-2-(1*H*-indol-3-yl)-4-(*p*-tolyl)pyrido[3,2-*e*][1,2,4]triazolo[4,3-*a*]pyrimidin-5 (7*H*)-one (**19d**). Yellow solid, (78% yield), mp 254–256 °C; IR (KBr): ν 3389 (NH), 1711, 1643 (2C=O), 1583 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 2.35 (s, 3H, CH₃), 2.45 (s, 3H, CH₃), 7.08–8.34 (m, 13H, Ar-H and pyridine-H), 8.73 (s, 1H, indole-H₂), 12.10 (br s, 1H, NH); MS, m/z (%) 589 (M⁺, 4), 397 (41), 279 (100), 236 (52), 193 (52), 43 (40). Anal. Calcd. for C₃₁H₂₁BrN₆O₂ (589.44): C, 63.17; H, 3.59; N, 14.26. Found: C, 63.09; H, 3.42; N, 14.17.

Ethyl2-(1*H*-Indol-3-yl)-5-oxo-7-phenyl-4-(*p*-tolyl)-5,7-dihydropyrido[3,2-*e*][1,2,4]triazolo[4,3-*a*]-pyrimidine-9-carboxylate (**19e**). Yellow solid, (75% yield), mp 212–214 °C; IR (KBr): ν 3342 (NH), 1718, 1644 (2C=O), 1579 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 1.29 (t, J = 7.1 Hz, 3H, CH₃), 2.34 (s, 3H, CH₃), 4.29 (q, J = 7.1 Hz, 2H, CH₂), 7.13–8.30 (m, 14H, Ar-H and pyridine-H), 8.73 (s, 1H, indole-H₂), 12.12 (br s, 1H, NH); MS, m/z (%) 540 (M⁺, 2), 521 (16), 361 (13), 270 (69), 167 (38), 91 (100). Anal. Calcd. for C₃₂H₂₄N₆O₃ (540.57): C, 71.10; H, 4.47; N, 15.55. Found: C, 71.07; H, 4.39; N, 15.37.

Ethyl2-(1*H*-Indol-3-yl)-5-oxo-4,7-di-*p*-tolyl-5,7-dihydropyrido[3,2-*e*][1,2,4]triazolo[4,3-*a*]pyrimidine-9-carboxylate (**19f**). Yellow solid, (77% yield), mp 238–240 °C; IR (KBr): ν 3348 (NH), 1746, 1673 (2C=O), 1576 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 1.30 (t, J = 7.1 Hz, 3H, CH₃), 2.23 (s, 3H, CH₃), 2.35 (s, 3H, CH₃), 4.26 (q, J = 7.1 Hz, 2H, CH₂), 7.08–8.32 (m, 13H, Ar-H and pyridine-H), 8.72 (s, 1H, indole-H₂), 12.09 (brs, 1H, NH); MS, m/z (%) 554 (M⁺, 4), 522 (24), 431 (100), 326 (88), 282 (25), 91 (10). Anal. Calcd. For C₃₃H₂₆N₆O₃ (554.60): C, 71.47; H, 4.73; N, 15.15. Found: C, 71.38; H, 4.70; N, 15.04.

Ethyl2-(1*H*-Indol-3-yl)-7-(4-methoxyphenyl)-5-oxo-4-(*p*-tolyl)-5,7 dihydropyrido[3,2-*e*][1,2,4]triazolo-[4,3-*a*]pyrimidine-9-carboxylate (**19g**). Yellow solid, (70% yield), mp 193–195 °C; IR (KBr): ν 3390 (NH), 1740, 1674 (2C=O), 1578 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 1.32 (t, J = 7.1 Hz, 3H, CH₃), 2.35 (s, 3H, CH₃), 3.82 (s, 3H, OCH₃), 4.34 (q, J = 7.1 Hz, 2H, CH₂), 7.03–8.34 (m, 13H, Ar-H and pyridine-H), 8.72 (s, 1H, indole-H₂), 12.10 (br s, 1H, NH); MS, m/z (%) 570 (M⁺, 10), 354 (52), 311 (62), 267 (85), 72 (54), 59 (100). Anal. Calcd. for C₃₃H₂₆N₆O₄ (570.60): C, 69.46; H, 4.59; N, 14.73. Found: C, 69.39; H, 4.48; N, 14.59.

Ethyl7-(4-Chlorophenyl)-2-(1*H*-indol-3-yl)-5-oxo-4-(*p*-tolyl)-5,7 dihydropyrido[3,2-*e*][1,2,4]triazolo-[4,3-*a*]pyrimidine-9-carboxylate (**19h**). Yellow solid, (78% yield), mp 243–245 °C; IR (KBr): ν 3383 (NH), 1742, 1675 (2C=O), 1577 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 1.31 (t, J = 7.1 Hz, 3H, CH₃), 2.35 (s, 3H, CH₃), 4.37 (q, J = 7.1 Hz, 2H, CH₂), 7.07–8.34 (m, 13H, Ar-H and pyridine-H), 8.72 (s, 1H, indole-H₂), 12.09 (brs, 1H, NH); MS, m/z (%) 575 (M⁺, 17), 352 (12), 293 (29), 126 (23), 72 (59), 59 (100). Anal. Calcd. For C₃₂H₂₃ClN₆O₃ (575.02): C, 66.84; H, 4.03; N, 14.62. Found: C, 66.69; H, 4.01; N, 14.47.

2-(1*H*-Indol-3-yl)-5-oxo-N,7-diphenyl-4-(*p*-tolyl)-5,7-dihydropyrido[3,2-*e*][1,2,4]triazolo [4,3-*a*]-pyrimidine-9-carboxamide (**19i**). Yellow solid, (82% yield), mp 268–270 °C; IR (KBr): ν

3385, 3213 (2NH), 1679, 1641(2C=O), 1598 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 2.35 (s, 3H, CH_3), 7.02–8.34 (m, 19H, Ar-H and pyridine-H), 8.72 (s, 1H, indole- H_2), 10.92 (br s, 1H, NH), 12.10 (br s, 1H, NH); MS, m/z (%) 587 (M^+ , 2), 441 (30), 271 (43), 158 (100), 128 (43), 91 (31). Anal. Calcd. for $\text{C}_{36}\text{H}_{25}\text{N}_7\text{O}_2$ (587.63): C, 73.58; H, 4.29; N, 16.69. Found: C, 73.52; H, 4.15; N, 16.57.

7-(4-Chlorophenyl)-2-(1*H*-indol-3-yl)-5-oxo-*N*-phenyl-4-(*p*-tolyl)-5,7-dihydropyrido[3,2-*e*][1,2,4]-triazolo[4,3-*a*]pyrimidine-9-carboxamide (**19j**). Yellow solid, (80% yield), mp 279–281 °C; IR (KBr): ν 3387, 3198 (2NH), 1683, 1642 (2C=O), 1597 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 2.34 (s, 3H, CH_3), 7.02–8.34 (m, 18H, Ar-H and pyridine-H), 8.72 (s, 1H, indole- H_2), 11.13 (br s, 1H, NH), 12.10 (br s, 1H, NH); MS, m/z (%) 622 (M^+ , 1), 341 (38), 267 (100), 129 (35), 98 (52), 57 (63). Anal. Calcd. for $\text{C}_{36}\text{H}_{24}\text{ClN}_7\text{O}_2$ (622.07): C, 69.51; H, 3.89; N, 15.76. Found: C, 69.42; H, 3.81; N, 15.59.

9-Acetyl-4-(4-chlorophenyl)-2-(1*H*-indol-2-yl)-7-phenylpyrido[3,2-*e*][1,2,4]triazolo[4,3-*a*]pyrimidin-5(7*H*)-one (**19k**). Yellow solid, (78% yield), mp 271–273 °C; IR (KBr): ν 3427 (NH), 1701, 1674 (2C=O), 1589 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 2.49 (s, 3H, CH_3), 7.21–8.10 (m, 14H, Ar-H and pyridine-H), 8.73 (s, 1H, indole- H_2), 12.04 (br s, 1H, NH); MS, m/z (%) 530 (M^+ , 100), 515 (53), 219 (47), 147 (87), 97 (21), 57 (79). Anal. Calcd. for $\text{C}_{30}\text{H}_{19}\text{ClN}_6\text{O}_2$ (530.96): C, 67.86; H, 3.61; N, 15.83. Found: C, 67.69; H, 3.54; N, 15.71.

9-Acetyl-4-(4-chlorophenyl)-2-(1*H*-indol-2-yl)-7-(*p*-tolyl)pyrido[3,2-*e*][1,2,4]triazolo [4,3-*a*]pyrimidin-5(7*H*)-one (**19l**). Yellow solid, (78% yield), mp 252–254 °C; IR (KBr): ν 3403 (NH), 1705, 1671 (2C=O), 1583 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 2.29 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 7.09–8.34 (m, 13H, Ar-H and pyridine-H), 8.73 (s, 1H, indole- H_2), 12.10 (br s, 1H, NH); MS, m/z (%) 544 (M^+ , 3), 362 (15), 303(100), 227 (27), 117 (39), 55 (21). Anal. Calcd. for $\text{C}_{31}\text{H}_{21}\text{ClN}_6\text{O}_2$ (544.99): C, 68.32; H, 3.88; N, 15.42. Found: C, 68.25; H, 3.69; N, 15.31.

Alternate Synthesis of **19a,e,i**

Equimolar amounts of 1-(1*H*-indol-3-yl)-3-(*p*-tolyl)prop-2-en-1-one (**3a**) (0.261 g, 1 mmol) and 7-amino-1-phenyl-[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1*H*)-one derivatives **20a,e,i** (1 mmol) in acetic acid (15 mL), was refluxed for 10 hours and then cooled to room temperature. The solid which was precipitated is collected, washed with water, dried, and recrystallized from DMF to give the corresponding products, **19a,e,i** which were identical in all respects (mp, mixed mp and IR spectra)

with those obtained from the reaction of thione **7a** with hydrazoneyl chlorides **8a,e,i** but the % yields are 69%, 67%, and 70%, respectively.

Synthesis of N'-(1-(1H-Indol-3-yl)ethylidene)-2-cyanoacetohydrazide (22)

To a solution of 2-cyanoacetohydrazide (**21**) (1.0 g, 10 mmol) and 3-acetyl-1H-indole (**1**) (0.159 g, 1 mmol) in ethanol (30 mL), acetic acid (2 mL) was added. The reaction mixture was heated under reflux for 3 hours and then left to cool. The solid product formed was collected by filtration, dried, and then crystallized from the appropriate solvent as yellow solid, (76% yield); mp 231–233 °C; IR (KBr): ν 3402 (NH), 2225 (CN) 1670 (C=O), 1602 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 2.57 (s, 3H, CH₃), 3.58 (s, 2H, CH₂), 7.13–7.23 (m, 2H, Ar-H), 7.47 (d, J = 6.9 Hz, 1H, Ar-H), 8.18 (d, J = 6.9 Hz, 1H, Ar-H), 8.29 (s, 1H, indole-H₂), 10.42 (br s, 1H, NH), 11.88 (br s, 1H, NH); MS, m/z (%) 240 (M⁺, 17), 170 (100), 153 (56), 183 (8), 125 (11), 70 (19), 55 (26). Anal. Calcd. for C₁₃H₁₂N₄O (240.26): C, 64.99; H, 5.03; N, 23.32. Found C, 64.85; H, 5.01; N, 23.22.

Synthesis of 3-(2-(1-(1H-Indol-3-yl)ethylidene)hydrazinyl)-2-cyano-3-oxo-N-phenylpropanethioamide (25)

To a stirred solution of potassium hydroxide (0.56 g, 10 mmol) in DMF (30 mL) was added with compound **22** (2.40 g, 10 mmol). After stirring for 30 minutes, phenyl isothiocyanate (**23**) (1.35 g, 10 mmol) was added to the resulting mixture. Stirring was continued overnight. The reaction mixture was acidified with HCl and the solid product was filtered off, washed with water, and dried. Recrystallization from EtOH produced a pure **25** as yellow solid, (70% yield); mp 143–145 °C; IR (KBr): ν 3402, 3387, 3182 (3NH), 2230 (CN) 1664 (C=O), 1600 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 2.57 (s, 3H, CH₃), 7.14–7.23 (m, 2H, Ar-H), 7.46 (d, J = 6.9 Hz, 1H, Ar-H), 8.18 (d, J = 6.9 Hz, 1H, Ar-H), 8.29 (s, 1H, indole-H₂), 9.85 (br s, 1H, NH), 10.40 (br s, 1H, NH), 11.88 (br s, 1H, NH), 13.11 (s, 1H, SH); MS, m/z (%) 375 (M⁺, 56), 207 (83), 165 (100), 119 (32), 77 (20). Anal. Calcd. for C₂₀H₁₇N₅OS (375.45): C, 63.98; H, 4.56; N, 18.65. Found C, 63.78; H, 4.48; N, 18.47.

Reaction of 25 with Hydrazoneyl Chlorides 8a,e,i

A mixture of **25** (0.375 g, 1 mmol) and N'-phenylbenzohydrazonyl chloride **8a,e,i** (1 mmol) in dioxane (30 mL) containing TEA (0.7 mL) was refluxed for 5 hours (monitored by TLC), allowed to cool and the solid formed was collected, washed with EtOH, dried, and recrystallized from DMF to give the respective 1,3,4-thiadiazole **27a–c**.

N'-(1-(1*H*-Indol-3-yl)ethylidene)-2-(5-acetyl-3-phenyl-1,3,4-thiadiazol-2(3*H*)-ylidene)-2-cyanoaceto- hydrazide(**27a**). Yellow solid, (68% yield); mp 191–193 °C; IR (KBr): ν 3425, 3385 (2NH), 2227 (CN), 1695, 1664(2C=O), 1608 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 2.47 (s, 3H, CH₃), 2.58 (s, 3H, CH₃), 7.19–8.25 (m, 9H, Ar-H), 8.68 (s, 1H, indole-H₂), 10.89 (br s, 1H, NH), 11.93 (br s, 1H, NH); $^{13}\text{C-NMR}$ (DMSO- d_6): 14.0, 24.5, 74.0, 116.4, 110.0, 116.7, 118.7, 121.4, 124.8, 128.4, 136.3, 130.5, 135.0, 143.4, 148.4, 157.4, 164.0, 189.9; MS, m/z (%) 442 (M⁺, 16), 364 (39), 275 (52), 215 (86), 107 (100), 81 (41), 43 (37). Anal. Calcd. For C₂₃H₁₈N₆O₂S (442.49): C, 62.43; H, 4.10; N, 18.99. Found C, 62.29; H, 4.03; N, 18.79.

Ethyl5-(2-(2-(1-(1*H*-Indol-3-yl)ethylidene)hydrazinyl)-1-cyano-2-oxoethylidene)-4-phenyl-4,5-dihydro-1,3,4-thiadiazole-2-carboxylate (**27b**). Yellow solid, (68% yield); mp 191–193 °C; IR (KBr): ν 3425, 3385 (2NH), 2227 (CN), 1695, 1664 (2C=O), 1608 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): δ 1.30 (t, J = 7.1 Hz, 3H, CH₃), 2.55 (s, 3H, CH₃), 4.27 (q, J = 7.1 Hz, 2H, CH₂), 7.10–8.29 (m, 9H, Ar-H), 8.69 (s, 1H, indole-H₂), 10.88 (br s, 1H, NH), 11.97 (br s, 1H, NH); $^{13}\text{C-NMR}$ (DMSO- d_6): MS, 14.0, 24.5, 74.0, 110.0, 110.5, 118.9, 120.4, 121.5, 125.1, 130.3, 130.5, 133.0, 143.4, 148.5, 157.0, 164.0, 189.8; m/z (%) 472 (M⁺, 15), 412 (37), 250(43), 139 (100), 108 (29), 43 (40). Anal. Calcd. for C₂₄H₂₀N₆O₃S (472.52): C, 61.00; H, 4.27; N, 17.79. Found C, 61.07; H, 4.17; N, 17.64.

5-(2-(2-(1-(1*H*-Indol-3-yl)ethylidene)hydrazinyl)-1-cyano-2-oxoethylidene)-N,4-diphenyl-4,5-dihydro-1,3,4-thiadiazole-2-carboxamide (**27c**). Yellow solid, (67% yield); mp 276–278 °C; IR (KBr): ν 3417, 3363, 3197(3NH), 2230 (CN), 1674, 1627 (2C=O), 1601 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6): 2.55 (s, 3H, CH₃), 7.06–8.28 (m, 14H, Ar-H), 8.47 (s, 1H, indole-H₂), 9.87 (br s, 1H, NH), 10.66 (br s, 1H, NH), 11.49 (br s, 1H, NH); MS, m/z (%) 519 (M⁺, 100), 372 (53), 266 (38), 137 (29), 120 (57), 43 (40). Anal. Calcd. For C₂₈H₂₁N₇O₂S (519.58): C, 64.73; H, 4.07; N, 18.87. Found C, 64.58; H, 4.03; N, 18.72.

Antitumor Activity Assay

The human carcinoma cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). These cells were grown on RPMI-1640 medium which is supplemented with 10% heat inactivated fetal calf serum, 1% L-glutamine, and 50 $\mu\text{g/mL}$ gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO₂ incubator and were sub-cultured for two to three times a week. For anti-tumor assays, the tumour cell lines were suspended in medium at concentration 5×10^4 cell/well in Corning® 96-well tissue culture plates, then incubated for 24 hours. The tested compounds were then added to 96-well plates (six

replicates) to achieve eight concentrations of each compound (started from 200 to 1.56 µg/mL). Six vehicle controls with media or 0.1% DMSO were run for each 96-well plate as a control. After 24 hours of incubation, the number of viable cells was determined by MTT assay. Therefore, the media were removed from the 96-well plate and replaced with 100 µL of fresh culture RPMI 1640 medium without phenol red and 10 µL of the 12 mM MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Co., St. Louis, MO, USA) stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96-well plates were then incubated at 37 °C with 5% of CO₂ for 4 hours. 85 µL aliquots of the media were removed from the wells, and 50 µL of DMSO was added to each well and mixed thoroughly using the pipette and incubated at 37 °C for 10 minutes. Then, the optical density was measured at 590 nm with the microplate reader to determine the number of viable cells and the percentage of viability was calculated as $[1 - (OD_t/OD_c)] \times 100\%$, where OD_t is the mean optical density of wells treated with the tested sample and OD_c is the mean optical density of untreated cells. The relation between surviving cells and drug concentration was plotted to get the survival curve of each tumour cell line after treatment with the specified compounds. The 50% Inhibitory Concentration (IC₅₀) and the concentration required to cause toxic effects in 50% of intact cells were estimated from graphic plots of the dose response curve for each concentration⁵⁵.

CONCLUSIONS

3-Acetylindole proved that it can be a useful precursor for synthesis of various 1,3-thiazoles, 1,2,4-thiadiazoles and pyrido[3,2-*e*][1,2,4]triazolo[4,3-*a*]pyrimidin-5(7*H*)-one. The structures of the newly synthesized compounds were confirmed by spectral data and elemental analyses. Some of the new compounds were tested *in vitro* against the MCF-7 human breast carcinoma cell line and compared with doxorubicin as the standard, using the MTT viability assay. Most of the tested compounds (2-(3-(1*H*-Indol-3-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-methyl-5-(phenyldiazenyl)thiazole, 2-(3-(1*H*-Indol-3-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-methyl-5-(*p*-tolyl diazenyl)thiazole, 2-(3-(1*H*-Indol-3-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-5-((4-chlorophenyl)diazenyl)-4-methyl-thiazole and 2-(3-(1*H*-Indol-3-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenyl-5-(phenyldiazenyl)thiazole) were found to have moderate to high anticancer activity.

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