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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]pyrimindin-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)ethlidene)-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 derivatives [1-4].Abundanceresearch 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, actingas potential anticancer agents[5,6].

Thiazoles are widely utilized in the development of drugs for treating allergies [7], as inhibitors of bacterial DNA gyrase B[8], sleep disorders[9],schizophrenia[10], inflammation[11], hypertension [12], pain [15], bacterial infections [16], and HIV [13]. It is also used as an inhibitor of bacterial DNA gyrase B [8] and as fibrinogen receptor antagonists[14].The 1,2,4-triazolopyrimidines attains 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 agent[17-22]. 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-hypertensive[23-32]. Despitethe 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 IC_{50} and IG_{50} [33-37]. 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, 2013[39]. 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 ofthe 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 agent [41]. Thiazoles can be established in drug development for the treatment of allergies [42], hypertension [43], inflammation[44],

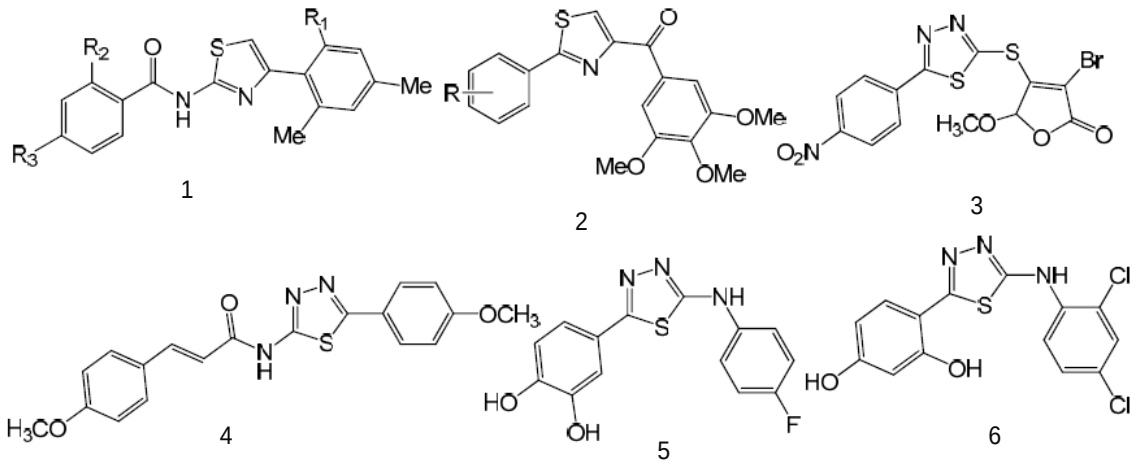
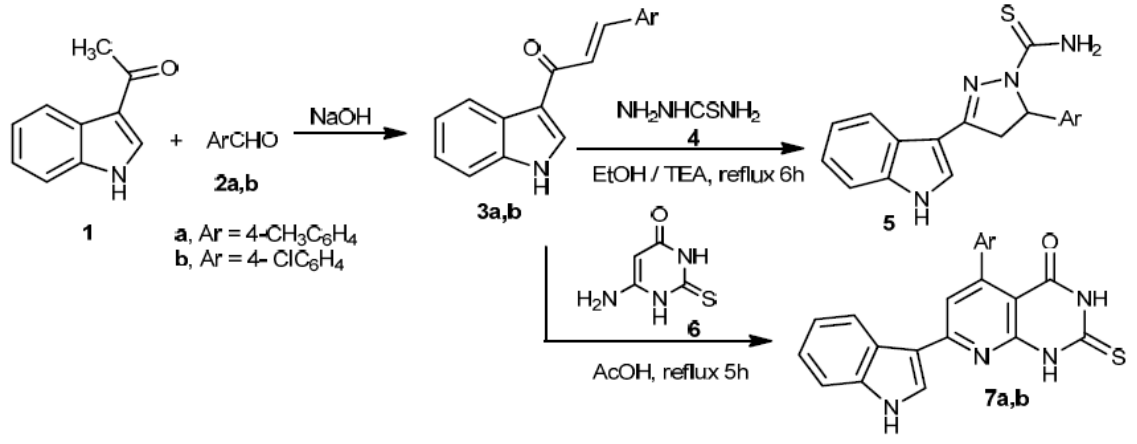


Figure 1. Lead compounds among thiazole and thiadiazole derivatives with anticancer activity [33-37]

Synthesis and Methodology

Chemistry

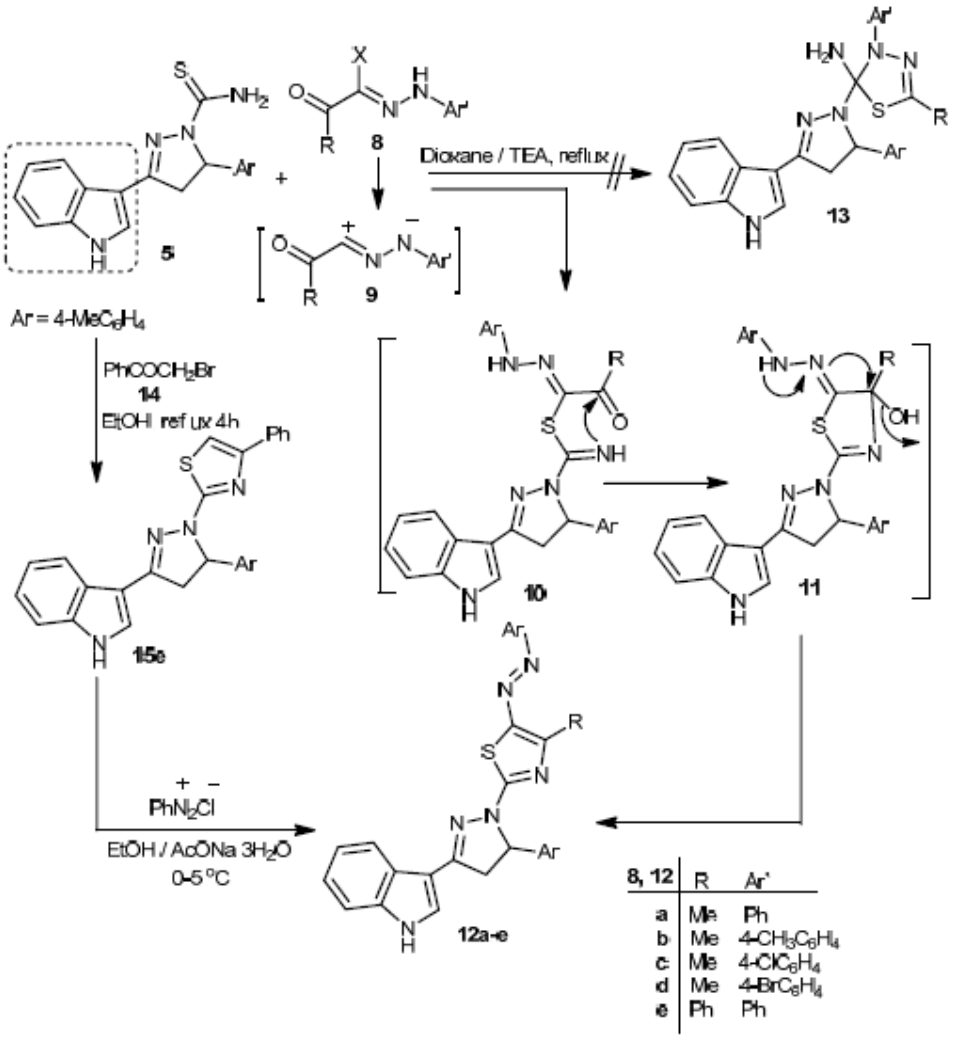
From the reaction of 3-actylinode (**1**) with 4-chlorobenzaldehyde (**2b**)and *p*-tolualdehyde (**2a**) respectively (Scheme 1), 3-(4-chlorophenyl)-1-(1*H*-indol-3-yl0prop-2-en-1-one (**3b**) [45] and 1-(1*H*-Indol-3-yl)-3-(*p*-tolyl)prop-2-en-1-one (**3a**) [46] were prepared as stated in the above literature.



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

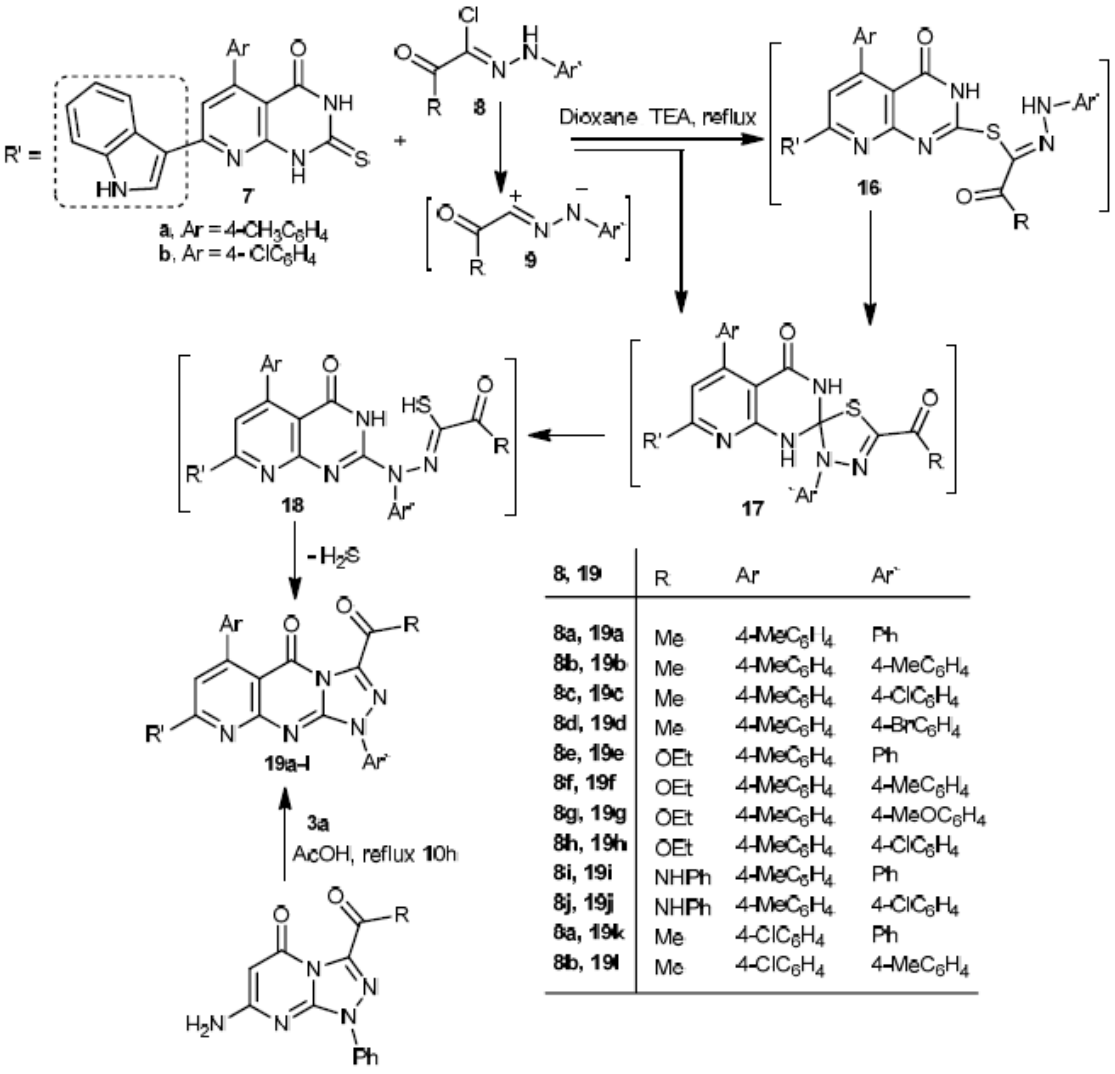
Treatment of 3-aryl-1-(1*H*-indol-3-yl)prop-2-en-1-ones **3a,b** with thiosemicarbazide (**4**) afforded 3-(1*H*-indol-2-yl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazole-1-carbthioamide (**5**). Similar treatment with 6-amino-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (**6**) generated 5-aryl-7-(1*H*-indol-3-yl)-2-thioxo2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-ones**7a,b**, respectively (Scheme 1). Structures **5** and **7** were expounded by chemical transformation, elemental analysis and spectral data.

Compounded **5** was reacted with the appropriate keto-hydrazonoyl 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 0ºC 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 thiohydrazonate**10**, which undergoes cyclization as soon as it formsan intermediate **11**. The latter loses one molecule of water to give the final product **12a**. Alternatively, 1,3-dipolar cycloadditon 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 hydrazonoyl chloride with 1-phenyl-1,4-dihydrotetrazole-5-thione[47] and 5-phenyl-1,3,4-thiadiazole-2(3*H*)-thione[48]. 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**was 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(1*H*)-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-l**, 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**.



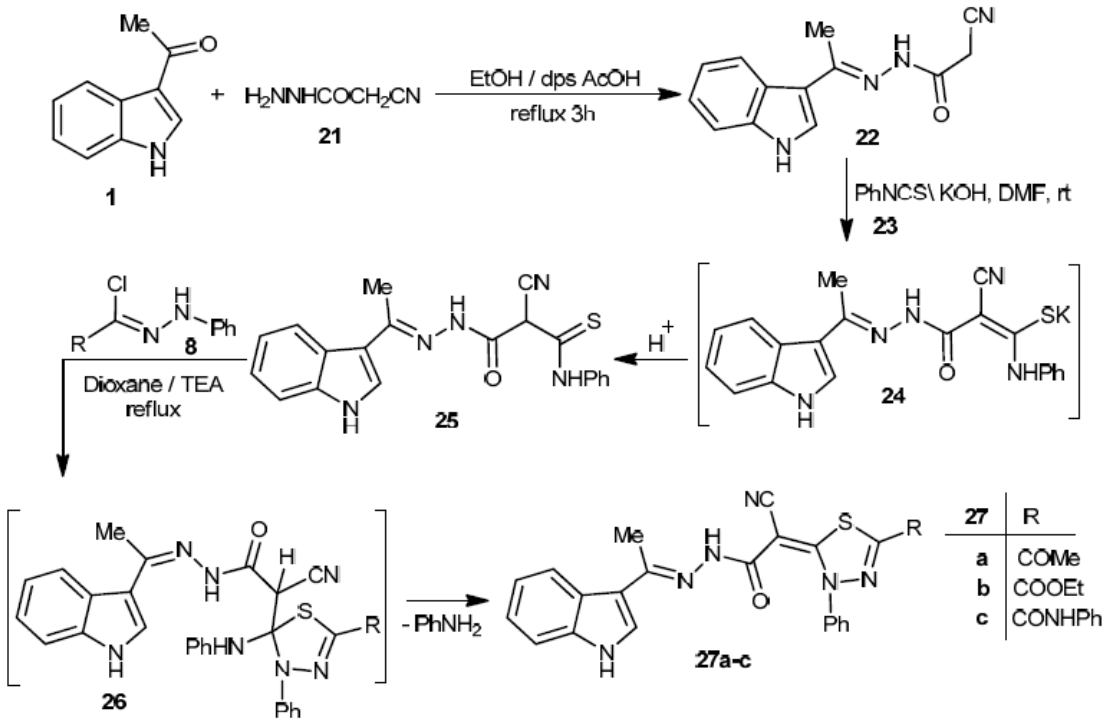
8, 19	R	Ar	Ar`
8a, 19a	Me	4-MeC6H4	Ph
8b, 19b	Me	4-MeC6H4	4-MeC6H4
8c, 19c	Me	4-MeC6H4	4-ClC6H4
8d, 19d	Me	4-MeC6H4	4-BrC6H4
8e, 19e	OEt	4-MeC6H4	Ph
8f, 19f	OEt	4-MeC6H4	4-MeC6H4
8g, 19g	OEt	4-MeC6H4	4-MeOC6H4
8h, 19h	OEt	4-MeC6H4	4-ClC6H4
8i, 19i	NHPh	4-MeC6H4	Ph
8j, 19j	NHPh	4-MeC6H4	4-ClC6H4
8a, 19k	Me	4-ClC6H4	Ph
8b, 19l	Me	4-ClC6H4	4-MeC6H4

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 thiohydrazonate 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 thiohydrazonate ester **16**, spiro intermediate **17** and thiohydrazide **18** did not succeed, even undermild 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 derivatives[50].

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.

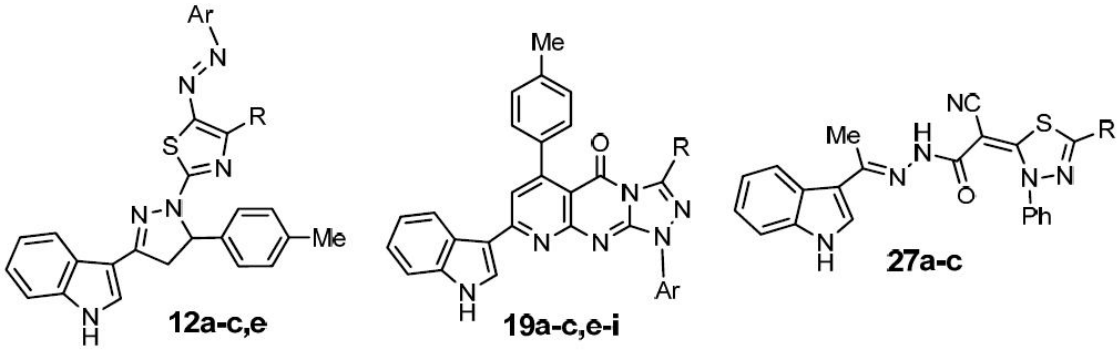
Biological Screening (Cytotoxic Activity)

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.Fromthe 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 $p < 0.001$.

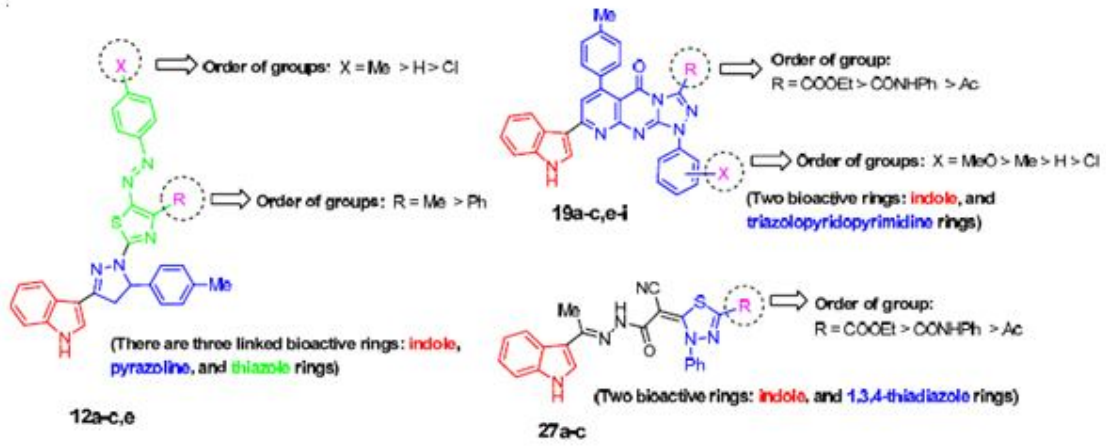
The results revealed that the tested compounds exhibit high variation in the inhibitory growth rates and activities against the tested tumour cell lines in a concentration-dependent manner compared to the reference drug as shown in Table 1 and Scheme 5.

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} (μM)
12a	CH_3	Ph	4.92
12b	CH_3	4-MeC_6H_4	0.95
12c	CH_3	4-ClC_6H_4	14.52
12e	Ph	Ph	19.44

Compound No.	R	Ar	IC_{50} (μM)
19a	CH_3CO	Ph	6.88
19b	CH_3CO	4-MeC_6H_4	4.68
19c	CH_3CO	4-ClC_6H_4	69.85
19e	CH_3CH_2OCO	Ph	4.83
19f	CH_3CH_2OCO	4-MeC_6H_4	5.49
19g	CH_3CH_2OCO	4-$MeOC_6H_4$	3.05
19h	CH_3CH_2OCO	4-ClC_6H_4	18.61
19i	PhNHCO	Ph	6.07
27a	CH_3CO	Ph	2.04
27b	CH_3CH_2OCO	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 triazolopyridopyrimide 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-the 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 ofan 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 (CO_2Et) 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 5are in the order of: COOEt>CONHPh> CH_3CO (**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 anNMR spectrometer operating at300 MHz (1H-NMR) and was run in deuterated dimethylsulfoxide (DMSO-d_6). Chemical shifts were relatedto 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 theliterature[51–54].

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

To a mixture of 1-(1*H*-indol-3-yl)-3-(*p*-tolyl)prop-2-en-1-one (**3a**) (2.61 g, 10 mmol) andthiosemicarbazide (0.92 g, 10 mmol) in EtOH (20 mL), a catalytic amount of triethylamine (1 mL) wasadded, then heated under reflux for 6 hours. The residual solid was collected, washed with EtOH andrecrystallized from acetic acid to give a pure **5** as a white solid (74%); mp 179–181 ºC; IR (KBr): *v* 3426,3212, 3175 (NH_2 and NH), 1569 (C=N) cm^{-1}; 1H-NMR (DMSO-d_6): δ 2.35 (s, 3H, CH_3), 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-H_2), 11.54 (br s, 2H, NH_2), 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 $C_{19}H_{18}N_4S$ (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 ºC; IR (KBr): *v* 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-H_2), 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 $C_{22}H_{16}N_4OS$ (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 ºC; IR (KBr): *v* 3463, 3368,3163 (3NH), 1678 (C=O), 1595 (C=N) cm^{-1};^{1}H-NMR (DMSO-d_6): δ 2.44 (s, 3H, CH_3), 4.70 (br s, 1H, NH), 7.10-8.32 (m, 9H, Ar-H and pyridine –H), 8.72 (s, 1H, indole-H_2), 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 $C_{21}H_{13}ClN_4OS$ (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-(1H-Indol-3-yl)-5-(p-tolyl)-4,5-dihydro-1H-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 ºC; IR (KBr): *v* 3409 (NH), 1642, 1593 (C=N)

cm^{-1}; ^{1}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); 13C-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 $C_{28}H_{24}N_6S$ (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*-tolyldiazenyl) thiazole (**12b**).Red solid, (72% yield); mp 193–195 ºC; IR (KBr): *v* 3403 (NH), 1642, 1590 (C=N) cm^{-1}; ^{1}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 $C_{29}H_{26}N_6S$ (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 ºC; IR (KBr): *v* 3403 (NH), 1642, 1590 (C=N) cm^{-1}; ^{1}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 $C_{28}H_{23}ClN_6S$ (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*-toslyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-5-((4-bromophenyl) diazenyl)-4-methyl-thiazole(**12d**). Red solid, (78% yield); mp 186–188 ºC; IR (KBr): *v* 3409 (NH), 1641, 1586 (C=N) cm^{-1}; ^{1}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 $C_{28}H_{23}BrN_6S$ (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 ºC; IR (KBr): *v* 3404 (NH), 1640, 1591 (C=N) cm^{-1}; ^{1}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-d_6): 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 $C_{33}H_{26}N_6S$ (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 in77% yield; mp 177–179 ºC; IR (KBr) *u* 3389 (CH), 1616 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ2.34 (s, 3H, CH_3), 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-H_5), 7.25–8.32 (m, 13H, Ar-H), 8.72 (s, 1H, indole-H_2), 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$C_{27}H_{22}N_4S$ (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 ºC 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 finallyrecrystallized from DMF to give a product that proved identical in all respects (mp, mixed mp and IRspectra) 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) indioxane (20 mL) were added TEA (0.14 mL, 1 mmol). The reaction mixture was refluxed until all ofthe starting materials had disappeared (8–12 hours, monitored by TLC). The solvent was

evaporated andthe residue was triturated with MeOH. The solid formed was collected and recrystallized from theappropriate solvent to give products **19a**–**l**. The products **19a**–**l** together with their physical constantsare 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 ºC; IR (KBr): *v* 3435 (NH), 1706, 1633 (2C=O), 1590 (C=N) cm^{-1};^{1}H-NMR (DMSO-d_6): δ 2.35 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 7.30–8.38 (m, 14H, Ar-H and pyridine-H),8.72 (s, 1H, indole-H_2), 12.02 (br s, 1H, NH); 13C-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 $C_{31}H_{22}N_6O_2$ (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**). Yellowsolid, (76% yield), mp 242–244 ºC; IR (KBr): *v* 3421 (NH), 1707, 1642 (2C=O), 1589 (C=N) cm^{-1};^{1}H-NMR (DMSO-d_6): δ 2.23 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 7.07-8.28 (m, 13H, Ar-Hand pyridine-H), 8.70 (s, 1H, indole-H_2), 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 $C_{32}H_{24}N_6O_2$ (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 ºC; IR (KBr): *v* 3386 (NH), 1710, 1644 (2C=O), 1589 (C=N)cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.35 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 7.09–8.34 (m, 13H, Ar-H andpyridine-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 $C_{31}H_{21}ClN_6O_2$ (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 ºC; IR (KBr): *v* 3389 (NH), 1711, 1643 (2C=O), 1583(C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.35 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 7.08–8.34 (m, 13H, Ar-H andpyridine-H), 8.73 (s, 1H, indole-H_2), 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 $C_{31}H_{21}BrN_6O_2$ (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 ºC; IR (KBr): *v* 3342 (NH), 1718, 1644 (2C=O),1579 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.29 (t, J = 7.1 Hz, 3H, CH_3), 2.34 (s, 3H, CH_3), 4.29 (q, J = 7.1Hz, 2H, CH_2), 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 $C_{32}H_{24}N_6O_3$(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 ºC; IR (KBr): *v* 3348 (NH), 1746, 1673 (2C=O), 1576 (C=N)cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.1 Hz, 3H, CH_3), 2.23 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 4.26(q, J = 7.1 Hz, 2H, CH_2), 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$C_{33}H_{26}N_6O_3$ (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 ºC; IR (KBr): *v* 3390 (NH), 1740,1674 (2C=O), 1578 (C=N) cm^{-1}; 1H-NMR (DMSO-d_6): δ 1.32 (t, J = 7.1 Hz, 3H, CH_3), 2.35 (s, 3H, CH_3),3.82 (s, 3H, OCH_3), 4.34 (q, J = 7.1 Hz, 2H, CH_2), 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 $C_{33}H_{26}N_6O_4$ (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 ºC; IR (KBr): *v* 3383 (NH), 1742,1675 (2C=O), 1577 (C=N) cm^{-1}; 1H-NMR (DMSO-d_6): δ 1.31 (t, J = 7.1 Hz, 3H, CH_3), 2.35 (s, 3H, CH_3),4.37 (q, J = 7.1 Hz, 2H, CH_2), 7.07-8.34 (m, 13H, Ar-H and pyridine-H), 8.72 (s, 1H, indole-H_2), 12.09 (brs, 1H, NH); MS, *m/z* (%) 575 (M^+, 17), 352 (12), 293 (29), 126 (23), 72 (59), 59 (100). Anal. Calcd. For$C_{32}H_{23}ClN_6O_3$ (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 ºC; IR (KBr): *v*

3385, 3213 (2NH), 1679, 1641(2C=O), 1598 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.35 (s, 3H, CH_3), 7.02–8.34 (m, 19H, Ar-H andpyridine-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 $C_{36}H_{25}N_7O_2$ (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 ºC; IR (KBr): *v* 3387, 3198 (2NH),1683, 1642 (2C=O), 1597 (C=N) cm^{-1}; ^{1}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 $C_{36}H_{24}ClN_7O_2$ (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 ºC; IR (KBr): *v* 3427 (NH), 1701, 1674 (2C=O), 1589 (C=N)cm^{-1}; ^{1}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 $C_{30}H_{19}ClN_6O_2$ (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 ºC; IR (KBr): *v* 3403 (NH), 1705, 1671 (2C=O),1583 (C=N) cm^{-1}; 1H-NMR (DMSO-d_6): δ 2.29 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 7.09–8.34 (m, 13H, Ar-Hand 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 $C_{31}H_{21}ClN_6O_2$(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) and7-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 hoursand 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 reactionof thione**7a** with hydrazonoyl 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-1*H*-indole (**1**) (0.159 g,1 mmol) in ethanol (30 mL), acetic acid (2 mL) was added. The reaction mixture was heated underreflux for 3 hours and then left to cool. The solid product formed was collected by filtration, dried, and thencrystallized from the appropriate solvent as yellow solid, (76% yield); mp 231–233 ºC; IR (KBr): *v* 3402(NH), 2225 (CN) 1670 (C=O), 1602 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d6): δ 2.57 (s, 3H, CH_3), 3.58 (s, 2H,CH_2), 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-H_2), 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 $C_{13}H_{12}N_4O$ (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 ºC; IR (KBr): *v*3402, 3387, 3182 (3NH), 2230 (CN) 1664 (C=O), 1600 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.57 (s, 3H,CH_3), 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 $C_{20}H_{17}N_5OS$ (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 Hydrazonoyl Chlorides **8a**,**e**,**i***

A mixture of **25** (0.375 g, 1 mmol) and N'-phenylbenzohydrazonoyl chloride **8a**,**e**,**i** (1mmol) indioxane (30 mL) containing TEA (0.7 mL) was refluxed for 5 hours (monitored by TLC), allowed to cooland the solid formed was collected, washed with EtOH, dried, and recrystallized from DMF to givethe 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 ºC; IR (KBr): *v* 3425, 3385 (2NH), 2227 (CN), 1695, 1664(2C=O), 1608 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.47 (s, 3H, CH_3), 2.58 (s, 3H, CH3), 7.19–8.25 (m,9H, Ar-H), 8.68 (s, 1H, indole-H_2), 10.89 (br s, 1H, NH), 11.93 (br s, 1H, NH); 13C-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$C_{23}H_{18}N_6O_2S$ (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-(1H-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 ºC; IR (KBr): *v* 3425, 3385 (2NH),2227 (CN), 1695, 1664 (2C=O), 1608 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.1 Hz, 3H, CH_3),2.55 (s, 3H, CH_3), 4.27 (q, J = 7.1 Hz, 2H, CH_2), 7.10–8.29 (m, 9H, Ar-H), 8.69 (s, 1H, indole-H_2), 10.88(br s, 1H, NH), 11.97 (br s, 1H, NH); ^{13}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 $C_{24}H_{20}N_6O_3S$ (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 ºC; IR (KBr): *v* 3417, 3363, 3197(3NH), 2230 (CN), 1674, 1627 (2C=O), 1601 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) : 2.55 (s, 3H, CH_3),7.06–8.28 (m, 14H, Ar-H), 8.47 (s, 1H, indole-H_2), 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$C_{28}H_{21}N_7O_2S$ (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 µg/mL gentamycin. The cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 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 x 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 the96-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 ºC with 5% of CO_2 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 ºC 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)]x 100%, where OD_t is the mean optical density of wells treated with the tested sample and OD_c is themean 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 (IC_{50}) 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 concentration [55].

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*-tolyldiazenyl)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)werefound to have moderate to high anticancer activity.

REFERENCES

1. Parmentier, J.G.; Poissonnet, G.; Goldstein, S. Practical Syntheses of 5-Trifluoromethyl-1H-indoles. Heterocycles 2002; 56: 465–476.
2. Black, D.S.; Craig, D.C.; Rezaie, R. Synthesis of mixed heterocalixarenes from benzofuranylmethanols and activated indoles. Chem. Commun. 2002; 8: 810–811.
3. Gaifa, L.; Wayne, K.N. Synthesis of novel indole analogues of mycophenolic acid as potential antineoplastic agents. Tetrahedron 2000; 56: 2583–2590.
4. Aiello, F.; Valacchi, G. Synthesis and Evaluation of Indole Based Molecules for Treatment of Oxidative Stress Related Diseases. Curr. Top. Med. Chem. 2014; 14: 2576–2589.
5. Broadbent, T.A.; Broadbent, H.S. The chemistry and pharmacology of indole-3-carbinol (indole-3-methanol) and 3-(methoxymethyl)indole. Part I. Curr. Med. Chem. 1998; 5: 337–352. [PubMed]
6. Riby, J.E.; Chang, G.H.; Firestone, G.L.; Bjeldanes, L.F. Ligand-independent activation of estrogen receptor function by 3,3’-diindolylmethane in human breast cancer cells. Biochem. Pharmacol. 2000; 60: 167–177.
7. Hargrave, K.D.; Hess, F.K.; Oliver, J.T. N-(4-Substituted-thiazolyl)oxamic acid derivatives, new series of potent, orally active antiallergy agents. J. Med. Chem. 1983; 26: 1158–1163.
8. Rudolph, J.; Theis, H.; Hanke, R.; Endermann, R.; Johannsen, L.; Geschke, F.U. Seco-Cyclothialidines: New concise synthesis, inhibitory activity toward bacterial and human DNA topoisomerases, and antibacterial properties. J. Med. Chem. 2001; 44: 619–626.
9. Ergenc, N.; Capan, G.; Gunay, N.S.; Ozkirimli, S.; Gungor, M.; Ozbey, S.; Kendi, E. Synthesis and hypnotic activity of new 4-thiazolidinone and 2-thioxo-4,5-imidazolidinedione derivatives. Arch. Pharm. Pharm. Med. Chem. 1999; 332: 343–347.
10. Jaen, J.C.;Wise, L.D.; Caprathe, B.W.; Tecle, H.; Bergmeier, S.; Humblet, C.C.; Heffner, T.G.; Meltzner, L.T.; Pugsley, T.A. 4-(1,2,5,6-Tetrahydro-1-alkyl-3-pyridinyl)-2-thiazolamines: A novel class of compounds with central dopamine agonist properties. J. Med. Chem. 1990; 33: 311–317.
11. Sharma, R.N.; Xavier, F.P.; Vasu, K.K.; Chaturvedi, S.C.; Pancholi, S.S. Synthesis of 4-benzyl-1,3-thiazole derivatives as potential anti-inflammatory agents: An analogue-based drug design approach. J. Enzym. Inhib. Med. Chem. 2009; 24: 890–897.
12. Patt, W.C.; Hamilton, H.W.; Taylor, M.D.; Ryan, M.J.; Taylor, D.G., Jr.; Connolly, C.J.C.; Doherty, A.M.; Klutchko, S.R.; Sircar, I.; Steinbaugh, B.A.; et al. Structure-activity relationships of a series of 2-amino-4-thiazole containing renin inhibitors. J. Med. Chem. 1992; 35: 2562–2572.

13. Bell, F.W.; Cantrell, A.S.; Hoegberg, M.; Jaskunas, S.R.; Johansson, N.G.; Jordon, C.L.; Kinnick, M.D.; Lind, P.; Morin, J.M.; Noreen, R.; et al. Phenethylthiazolethiourea (PETT) compounds, a new class of HIV-1 reverse transcriptase inhibitors. 1. Synthesis and basic structure-activity relationship studies of PETT analogs. J. Med. Chem. 1995; 38: 4929–4936.
14. Badorc, A.; Bordes, M.F.; de Cointet, P.; Savi, P.; Bernat, A.; Lale, A.; Petitou, M.; Maffrand, J.P.; Herbert, J.M. New orally active non-peptide fibrinogen receptor (GpIIb-IIIa) antagonists: Identification of ethyl 3-[N-[4-[4-amino[(ethoxycarbonyl) imino]methyl]phenyl]-1,3-thiazol-2-yl]-N-[1-(ethoxycarbonyl)methyl] piperid-4-yl]amino]propionate (SR 121787) as a potent and long-acting antithrombotic agent. J. Med. Chem. 1997; 40: 3393–3401.
15. Carter, J.S.; Kramer, S.; Talley, J.J.; Penning, T.; Collins, P.; Graneto, M.J.; Seibert, K.; Koboldt, C.; Masferrer, J.; Zweifel, B. Synthesis and activity of sulfonamide-substituted 4,5-diaryl thiazoles as selective cyclooxygenase-2 inhibitors. Bioorg. Med. Chem. Lett. 1999; 9: 1171–1174.
16. Tsuji, K.; Ishikawa, H. Synthesis and anti-pseudomonal activity of new 2-isocephems with a dihydroxypyridone moiety at C-7. Bioorg. Med. Chem. Lett. 1994; 4: 1601–1606.
17. Astakhov, A.V.; Chernyshev, V.M. Molecular structure of 3-amino[1,2,4]triazolo-[4,3-a] pyrimidin-5-one in various tautomeric forms: Investigation by DFT and QTAIM methods. Chem. Heterocycl. Compd. 2014; 50: 319–326.
18. Gomha, S.M. A facile one-pot synthesis of 6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-d]-1,2,4-triazolo [4,5-a]pyrimidin-5-ones. Monatshefte Chem. 2009; 140: 213–220.
19. Fares, M.; Abou-Seri, S.M.; Abdel-Aziz, H.A.; Abbas, S.E.S.; Youssef, M.M.; Eladwy, R.A. Synthesis and antitumor activity of pyrido[2,3-d]pyrimidine and pyrido[2,3-d] [1,2,4]triazolo[4,3-a]pyrimidine derivatives that induce apoptosis through G(1) cell-cycle arrest. Eur. J. Med. Chem. 2014; 83: 155–166.
20. Gomha, S.M.; Ahmed, S.A.; Abdelhamid, A.O. Synthesis and cytotoxicity evaluation of some novel thiazoles, thiadiazoles, and pyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidin-5(1H)-one incorporating triazole moiety. Molecules 2015; 20: 1357–1376.
21. Liu, X.H.; Sun, Z.H.; Yang, M.Y.; Tan, C.X.; Weng, J.Q.; Zhang, Y.G.; Ma, Y. Microwave assistant one pot synthesis, crystal structure, antifungal activities and 3D-QSAR of novel 1,2,4-triazolo[4,3-a]pyridines. Chem. Biol. Drug Des. 2014; 84: 342–347.
22. Gomha, S.M.; Badrey, M.G. Ecofriendlyregioselective one-pot synthesis of chromeno[4,3-d] [1,2,4]triazolo [4,3-a]pyrimidine. Eur. J. Chem. 2013; 4: 180–184.

23. Zhang, L.J.; Yang, M.Y.; Sun, Z.H.; Tan, C.X.;Weng, J.Q.;Wu, H.K.; Liu, X.H. Synthesis and antifungal activity of 1,3,4-thiadiazole derivatives containing pyridine group. Lett. Drug Des. Discov. 2014; 11: 1107–1111.
24. Gomha, S.M.; Khalil, K.D.; El-Zanate, A.M.; Riyadh, S.M. A facile green synthesis and anti-cancer activity of bis-arylhydrazononitriles, triazolo[5,1-c][1,2,4]triazine, and 1,3,4-thiadiazoline. Heterocycles2013; 87: 1109–1120.
25. Yan, S.L.; Yang, M.Y.; Sun, Z.H.; Min, L.J.; Tan, C.X.;Weng, J.Q.;Wu, H.K.; Liu, X.H. Synthesis and antifungal activity of 1,2,3-thiadiazole derivatives containing 1,3,4-thiadiazole moiety. Lett. Drug Des. Discov. 2014; 11: 940–943.
26. Tong, J.Y.; Sun, N.B.; Wu, H.K.; Liu, X.H. Synthesis, crystal structure and biological activity of N-(5-(Otolyl)-1,3,4-thiadiazol-2-yl)cyclopropanecarboxamide. J. Chem. Soc. Pak. 2013; 35: 1349–1353.
27. Gomha, S.M.; Salah, T.A.; Abdelhamid, A.O. Synthesis, characterization and pharmacological evaluation of some novel thiadiazoles and thiazoles incorporating pyrazole moiety as potent anticancer agents. Monatshefte Chem. 2015; 146: 149–158.
28. Li, Z.;Wang, X.; Da, Y. Synthesis of 2-(5-(2-chlorophenyl)-2-furoylamino)-5-aryloxymethyl-1,3,4-thiadiazoles under microwave irradiation. Synth. Commun. 2001; 31: 1829–1836.
29. Gomha, S.M.; Abdel-Aziz, H.A. Synthesis of new heterocycles derived from 3-(3-methyl-1H-indol-2-yl)-3-oxopropanenitrile as potent antifungal agents. Bull. Korean Chem. Soc. 2012; 33: 2985–2990.
30. Liu, X.; Shi, Y.; Ma, Y.; Zhang, C.; Dong, W.; Pan, L.; Wang, B.; Li, Z. Synthesis, antifungal activities and 3D-QSAR study of N-(5-substituted-1,3,4-thiadiazol-2-yl)cyclopropanecarboxamides. Eur. J. Med. Chem. 2009; 44: 2782–2786.
31. Ahmad, T.; Singh, A.K.; Jaiswal, N.; Singh, D. Synthesis and pharmacological activity of 1,3,4-thiadiazole derivatives: A review. Int. Res. J. Pharm. 2012; 3: 70–82.
32. Gomha, S.M.; Riyadh, S.M. Synthesis under microwave irradiation of [1,2,4]triazolo[3,4-b] [1,3,4]thiadiazoles and other diazoles bearing indole moieties and their antimicrobial evaluation. Molecules 2011; 16: 8244–8256.
33. Qiu, X.L.; Li, G.; Wu, G.; Zhu, J.; Zhou, L.; Chen, P.L.; Chamberlin, A.R.; Lee, W.H. Synthesis and biological evaluation of a series of novel inhibitor of Nek2/Hec1 analogues. J. Med. Chem. 2009; 52: 1757–1767.
34. Tsoua, H.; MacEwan, G.; Birnberg, G.; Grosu, G.; Bursavich, M.G.; Bard, J.; Brooijmansa, N.; Toral-Barzab, L.; Hollanderb, I.; Mansoura, T.S.; et al. Discovery and optimization of 2-(4-substituted-pyrrolo [2,3-b] pyridin-3-yl)methylene-4-hydroxybenzofuran-3(2H)-ones as

potent and selective ATP-competitive inhibitors of the mammalian target of rapamycin (mTOR). Bioorg. Med. Chem. Lett. 2010; 20: 2321–2325.

35. Mavrova, A.T.; Wesselinova, D.; Tsenov, Y.A.; Denkova, P. Synthesis, cytotoxicity and effects of some 1,2,4-triazole and 1,3,4-thiadiazole derivatives on immunocompetent cells. Eur. J. Med. Chem. 2009; 44: 63–69.
36. Oleson, J.J.; Slobada, A.; Troy, W.P.; Halliday, S.L.; Landes, M.J.; Angier, R.B.; Semb, J.; Cyr, K.; Williams, J.H. The carcinostatic activity of some 2-amino-1,3,4-thiadiazoles. J. Am. Chem. Soc. 1955; 77: 6713–6714.
37. Matysiak, J.; Opolski, A. Synthesis and antiproliferative activity of N-substituted 2-amino-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles. Bioorg. Med. Chem. 2006; 14: 4483–4489.
38. Anupriya, S.;Sushil,S.-K. Synthesis of 1-Aryloxy-2-hydroxy-3-substituted Aminocyclohexane and its Analogues for Possible Antimalarial Activity. International Journal of Scientific Research and Reviews, 2013; 2(4): 13- 21.
39. Meenachi, S.;Chitra, S. A Review of Chemistry and Biological Importance of Schiff Base. International Journal of Scientific Research and Reviews. 2014; 3(1): 08- 18.
40. Abdelhamid, A. O.;Gomha, S. M.;Abdelriheem, N.A.; Kandeel, S. M. Synthesis of New 3-Heteroarylindoles as Potential Anticancer Agents. Molecules 2016; 21: 929.
41. Broadbent, T.A.; Broadbent, H.S. The chemistry and pharmacology of indole-3-carbinol (indole-3-methanol) and 3-(methoxymethyl)indole. Part I. Curr. Med. Chem. 1998; 5: 337–352.
42. Hargrave, K.D.; Hess, F.K.; Oliver, J.T. N-(4-Substituted-thiazolyl)oxamic acid derivatives, new series of potent, orally active antiallergy agents. J. Med. Chem. 1983; 26: 1158–1163.
43. Patt, W.C.; Hamilton, H.W.; Taylor, M.D.; Ryan, M.J.; Taylor, D.G., Jr.; Connolly, C.J.C.; Doherty, A.M.; Klutchko, S.R.; Sircar, I.; Steinbaugh, B.A.; et al. Structure-activity relationships of a series of 2-amino-4-thiazole containing renin inhibitors. J. Med. Chem. 1992; 35: 2562–2572.
44. Sharma, R.N.; Xavier, F.P.; Vasu, K.K.; Chaturvedi, S.C.; Pancholi, S.S. Synthesis of 4-benzyl-1,3-thiazole derivatives as potential anti-inflammatory agents: An analogue-based drug design approach. J. Enzym. Inhib. Med. Chem. 2009; 24: 890–897.
45. Manna, F.; Chimenti, F.; Bolasco, A.; Bizzarri, B.; Filippelli, W.; Filippelli, A.; Gagliardi, L. Anti-inflammatory, analgesic and antipyretic 4,6-disubstituted 3-cyano-2-aminopyridines. Eur. J. Med. Chem. 1999; 34: 245–254.

46. Ismail, M.M.F.; Khalifa, N.M.; Fahmy, H.H.; Nossier, E.S.; Abdulla, M. Design, docking, and synthesis of some new pyrazoline and pyranopyrazole derivatives as anti-inflammatory agents. J. Heterocycl. Chem. 2014; 51: 450–458.
47. Butler, R.N. Comprehensive Heterocyclic Chemistry; Katritzky, A.R., Rees, C.W., Scriven, E.F.V., Eds.; Pergamon Press: New York, NY, USA, 1996; 4: 621–678.
48. Huisgen, R.; Grashey, R.; Seidel, M.; Knupfer, H.; Schmidt, R. 1,3-Dipolare additionen, III. Umsetzungen des diphenylnitriliminsmit carbonyl und thiocarbonyl-verbindungen. Justus Liebigs Ann. Chem. 1962; 658: 169–180.
49. Mosselhi, M.A.N. A convenient synthesis of novel derivatives of pyrido[2,3-d][1,2,4]triazolo[4,3-a] pyrimidine-5,6-dione. Monatshefte Chem. 2002, 133, 1297–1304.
50. Eweiss, N.F.; Osman, A. Synthesis of heterocycles-2. New routes to acetylthiadiazolines and arylazothiazoles. J. Heterocycl. Chem. 1980; 17: 1713–1717.
51. Shawali, A.S.; Osman, A. Reaction of dimethylphenacylsulfonium bromide with N-nitrosoacetaryl-amides and reactions of the products with nucleophiles. Bull. Chem. Soc. Jpn. 1976; 49: 321–324.
52. Shawali, A.S.; Osman, A. Synthesis and reactions of phenylcarbamoyl-arylhydrazidic chlorides. Tetrahedron 1971; 27: 2517–2528.
53. Asiri, A.M.; Zayed, M.E.M.; Ng, S.W. Ethyl (Z)-2-chloro-2-(2-phenylhydrazin-1-ylidene)acetate. ActaCrystallogr. 2011; 67: 1962.
54. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods 1983; 65: 55–63.
55. Fu, L.; Feng, X.; Wang, J.-J.; Xun, Z.; Hu, J.-D.; Zhang, J.-J.; Zhao, Y.-W.; Huang, Z.-B.; Shi, D.-Q. Efficient Synthesis and Evaluation of Antitumor Activities of Novel Functionalized 1,8-Naphthyridine Derivatives. ACS Comb. Sci. 2015; 17: 24–31.