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Molecular Modeling and Synthesis of New Heterocyclic Compounds Containing Pyrazole as Anticancer Drugs

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Abstract

Several modifications in CA-4 were reported in literature for the development of various tubulin inhibitors. In our study, twenty-two newly synthesized heterocyclic derivatives of Combretastatin A-4 (CA-4) have been tested for their cytotoxic effect on four different types of cells with malignant behavior using CA-4 as a positive control. Compounds **5b**, **15** and **16** showed the foremost potent antiproliferative activities as compared to CA-4 with IC50 starting from 6.9 to 13.7 μM. Molecular docking was performed with the crystal structure of tubulin employing a potent tubulin inhibitor CA-4 as a parent molecule. Molecular study advised that **5b**, **15**, **16** and **17** are promising tubulin inhibitors.

Keywords

Tubulin, CA-4, Anticancer, Pyrazole, Molecular Modeling

1. Introduction

Microtubule-targeting agents are used clinically as antitumor drugs. Their anticancer activity is commonly due to tubulin inhibition. They prevent the formation of microtubule, thus affecting cellular replication and shape [1]. In general, antitubulin agents bind with five totally different binding pockets on the β-tubulin subunit [2]. Agents that strongly react with colchicine binding site induce depolymerization of tubulin, therefore they inhibit tubulin action [3] (**Figure 1(a)**). This study will focus on agents that target colchicine-binding site [4].

The natural product CA-4 that is under investigation as it has a strong affinity to colchicine binding site, and therefore a strong tubulin inhibitor. Thus, we use

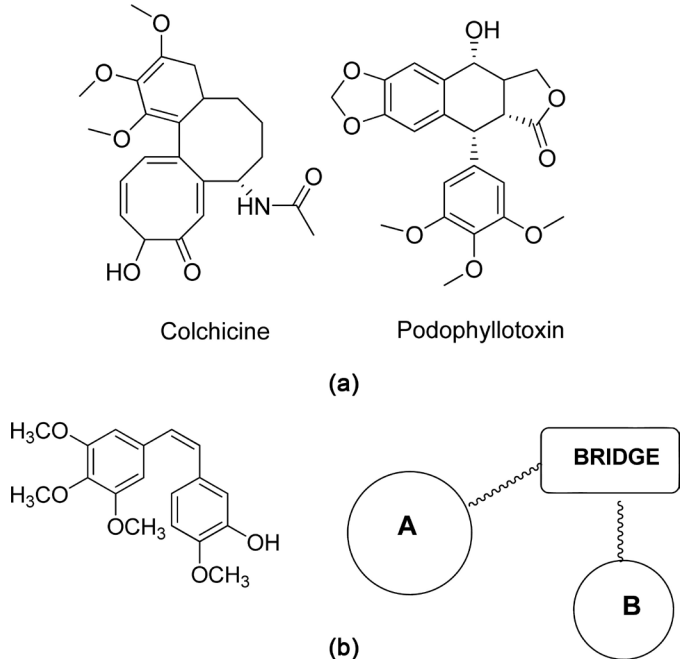


Figure 1. (a) Tubulin inhibitors that bind to colchicine binding site; (b) Common structure characteristic of CA-4.

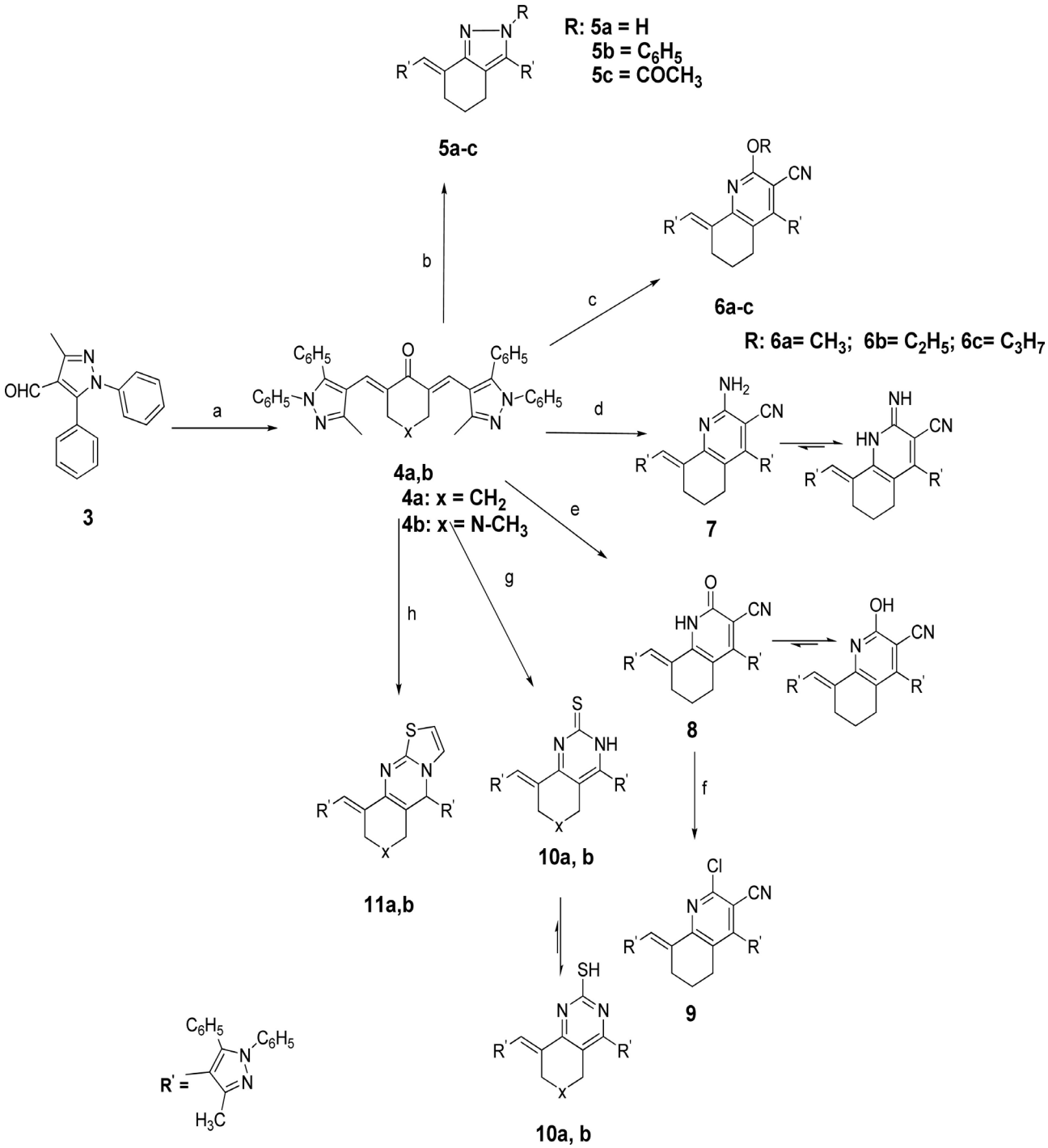
CA-4 as a lead molecule for synthesis of new derivatives and more studied as tubulin inhibitors [5]. It is formed of three parts (2 carbons bridge, ring A and ring B) [6] (**Figure 1(b)**). Various changes to CA-4 structure have been performed in literature. The changes to the central bridge affect the cytotoxic activity the most [7]-[15]. Ring A has received little or no attention while ring B has received larger attention. Impressed by these finding, the double bond of CA-4 is substituted by *α*, *β*-unsaturated ketone, pyrazole, quinoline-3-carbonitriles, fused pyrimidine, fused quinazoline moieties, imine derivatives and 2-amino-4*H*-chromenes. Besides, ring A and B are replaced by 1, 5-diphenylpyrazole, naphthalene and pyrazole moieties. Those were evaluated in *vitro* for their anticancer tendency toward four cancer cell types. Additionally, molecular docking studies are performed and compared to biological results.

2. Materials and Methods

2.1. Chemistry

The new compounds are prepared by different pathways described in **Scheme 1** and **Scheme 2**. The starting compound **3** was obtained *via* Vilsmeir Haack's formylation of the pyrazole derivatives using $POCl_3$/DMF [16]. The *α*, *β* unsaturated carbonyl derivatives **4a**, **4b** were synthesized *via* Claisen-Schmidt condensation [17] of cyclic ketones **4a**, **b** with substituted pyrazole-4-carbaldehyde **3** in an ethanolic solution of sodium hydroxide [18] [19] [20] [21] [22]. Cyclocondensation of enone **4a** with hydrazine hydrate or phenyl hydrazine using ethanol or glacial ethanoic acid afforded pyrazoles **5a-c** [23].

2-Substituted quinoline-3-carbonitrile products were made by the reaction of

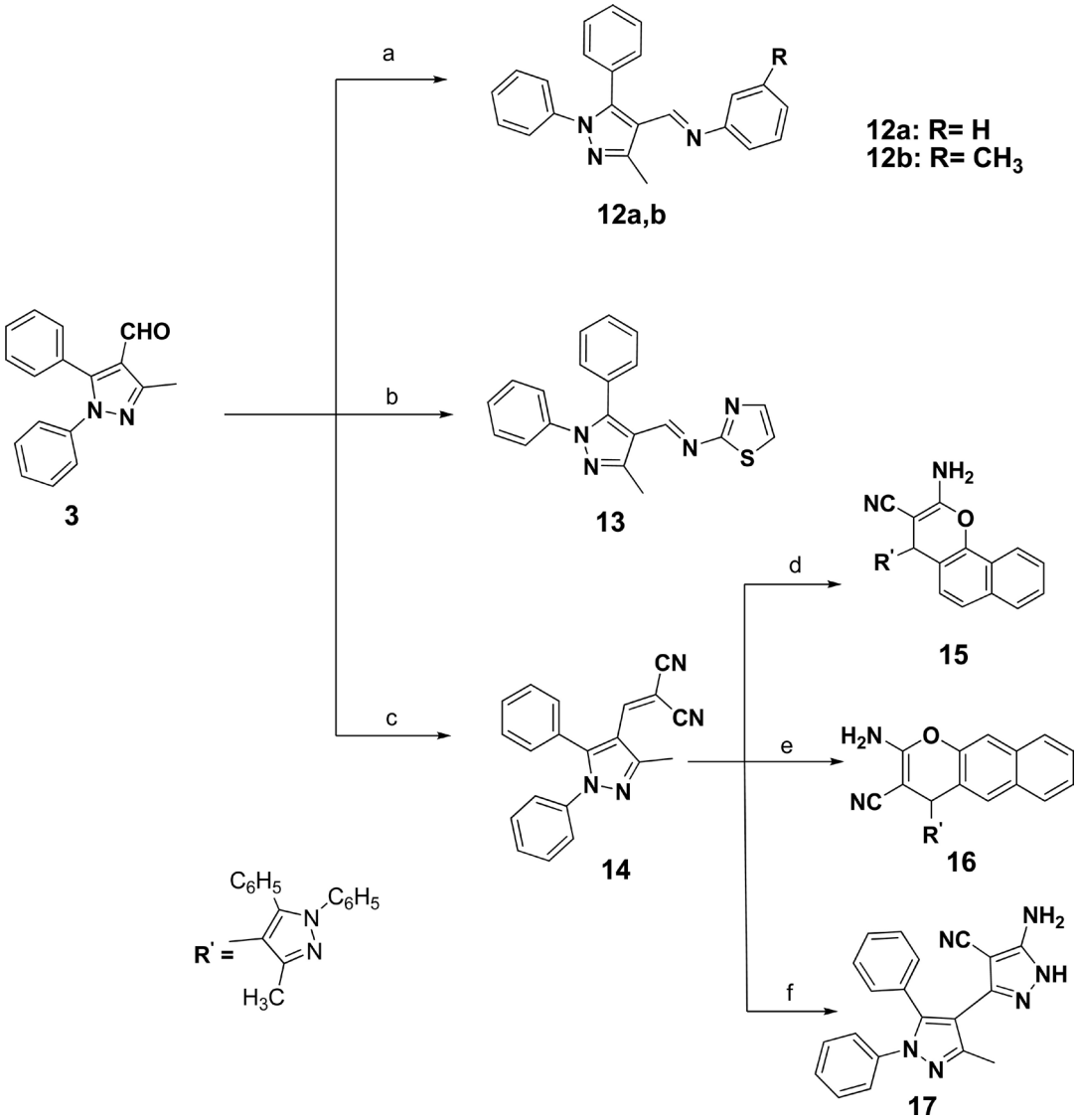


Scheme 1. Reagents and conditions: (a) cyclohexanone or N-methyl piperidone, alchoholic NaOH; (b) NH_2NHR, absolute ethanol or NH_2NH_2, AcOH; (c) malononitrile, sodium methoxide, sodium ethoxide or sodium propoxide, methanol, ethanol or n-propanol; (d) malononitrile, ammonium acetate, absolute ethanol; (e) ethylcyanoacetate, ammonium acetate, absolute ethanol; (f) $POCl_3$\DEA; (g) thiourea, Na metal, butanol; (h) 2-aminothiazole, AcOH.

malononitrile with compounds **4a** in sodium alkoxide to give the corresponding 2-alkoxyquinoline-3-carbonitriles derivatives **6a-c**. Furthermore, a reaction of chalcone **4a** with malononitrile and ammonium acetate in ethanol gave 2-aminoquinoline-3-carbonitrile derivative **7**. Also, 2-oxoquinoline-3-carbonitrile

8 was successfully synthesized by cyclocondensation reaction of compound **4a** with ethylcyanoacetate and ammonium acetate in absolute ethanol. In another hand, chloro product **9** was collected by the reaction of compound **8** with $POCl_3$ in catalytic amount of *N, N*-diethylaniline (DEA). Quinazoline-2-thione **10a**, pyrido[4,3-*d*]pyrimidine-2-thione analogue **10b** were obtained by reaction of chalcone **4a,b** with thiourea in refluxing Na*OBu* in butyl alcohol [21]. Moreover, new derivatives of thiazolo[2,3-*b*]quinazoline **11a** and thiazolo[3,2-*a*]pyrimidine **11b** were prepared by reacting 2-aminothiazole with the enones **4a,b** in glacial ehanoic acid [18].

Other targeted imino derivatives **12a**, **b** and **13** were prepared *via* condensation of the pyrazole carbaldehyde **3** with the appropriate aromatic amine in glacial ethanoic acid [24] [25] (Scheme 2). Additionally, substituted malononitrile



Scheme 2. Reagents and conditions: (a) aniline or *m*-toluidine, AcOH; (b) 2-aminothiazole, AcOH; (c) malononitrile, absolute ethanol; (d) *α*-naphthol, methansulfonic acid; (e) *β*-naphthol, methansulfonic acid; (f) NH_2NH_2, absolute ethanol.

nol. 2-Amino-4*H*-chromenes **15** and **16** were prepared from condensation of **14** was prepared from condensation of compound **3** with malononitrile in ethamalononitrile derivative **14** with naphthols using methanesulfonic acid [26]. Also, it was found that 5-amino-4-cyanopyrazole analogue **17** resulted when two moles of malononitrile derivative **14** condensed with one mole of hydrazine hydrate in absolute ethanol. All the analytical and spectroscopic data of the prepared compounds were fully accordance with their represented structures.

2.2. Biology

Four neoplastic cell types namely; (MCF-7), (HCT-116), (Hela) and (PC3) are obtained through VACSERA, Cairo, Egypt. Combretastatin A-4 was used as reference for judgment on anticancer activity of newly synthesized molecules.

The reagents RPMI-1640 medium, MTT, DMSO and CA-4 (sigma co., St. Louis, USA), Fetal Bovine blood serum (GIBCO, UK) [27].

The MTT assay was applied to assess cytotoxic activity using the cell lines mentioned above. This type of colorimetrical chemical assay relies on the modification of the yellow tetrazolium bromide (MTT) to a color of formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Cell lines were cultured in RPMI-1640 medium with ten percent fetal bovine blood serum. Then, antibiotics added were one-hundred units/ml penicillin and one-hundred μg/ml streptomycin at 37˚C in a five percent CO_2 incubator. Then, drugs were prepared in different concentrations and added to the cells, incubated for 24 hrs. After the period was completed, MTT solution (20 μl) with concentration 5 mg/ml was added to the mixture and incubated for 4 hrs. Then, DMSO (100 μl) is sided to every single plate to remove purple color of formazan. The colorimetrical chemical assay is measured and recorded at an absorbance of 570 nm employing a plate reader (EXL 800, USA) [28] [29].

2.3. Molecular Modeling

The docking studies and modeling calculations were done using MOE version 2008.10 release of Chemical Computing Groups using Windows XP software. The 3D structures of the synthesized molecules were built using Chem Biooffice suite then saved as Mol_2 format with the help of MOE software, then subjected to docking simulation. The three dimensional X-ray structure of protein (PDB code: 1SA1) was obtained from the Protein Data Bank.

3. Results

3.1. Antitumor Activity

All the synthesized compounds were evaluated for their cytotoxic activity toward a panel of 4 human neoplastic cell lines using the MTT assay. The results, in Table 1 showed that every compound have antitumor activities with noticeable differences due to structural variations.

As shown in Table 1, compound **5b** with 2-phenyl substituted indazole

Table 1. Cytotoxic activity (IC50, μg/ml) of (4-17).

Compounds	In vitro Cytotoxicity IC50 (μg/ml)					
	X	**R**	**HCT-116**	**Hela**	**PC3**	**MCF-7**
CA-4			**10.5 ± 0.73**	**9.4 ± 0.61**	**20.2 ± 1.12**	**5.3 ± 0.42**
4a	CH_2		58.7 ± 3.65	61.9 ± 3.42	53.0 ± 2.98	55.4 ± 3.11
4b	N-CH_3		27.6 ± 1.98	20.1 ± 1.60	26.2 ± 1.82	**17.1 ± 1.64**
5a			40.8 ± 2.73	33.5 ± 2.06	41.6 ± 2.21	37.5 ± 2.30
5b		Ph	**8.6 ± 0.51**	**8.2 ± 0.73**	**12.7 ± 0.88**	**6.9 ± 0.51**
5c		$COCH_3$	83.8 ± 4.73	>100	>100	84.9 ± 3.99
6a		CH_3	22.6 ± 1.87	**14.9 ± 1.23**	**17.7 ± 1.41**	**14.0 ± 1.41**
6b		C_2H_5	29.3 ± 2.11	38.9 ± 2.25	28.5 ± 1.69	**20.4 ± 1.85**
6c		C_3H_7	**19.8 ± 1.50**	**16.0 ± 1.32**	**18.9 ± 1.39**	**13.8 ± 1.42**
7			63.4 ± 3.98	72.6 ± 3.83	74.5 ± 3.85	62.3 ± 3.35
8			52.6 ± 3.32	80.4 ± 4.05	65.8 ± 3.37	51.1 ± 2.89
9			70.6 ± 4.52	94.1 ± 4.53	97.3 ± 4.60	80.1 ± 3.74
10a	CH_2		35.3 ± 2.24	50.5 ± 2.89	46.7 ± 2.38	31.8 ± 2.15
10b	N-CH_3		92.9 ± 4.95	>100	93.1 ± 4.12	89.5 ± 4.32
11a	CH_2		96.4 ± 5.24	86.0 ± 4.19	82.9 ± 3.93	>100
11b	N-CH_3		65.4 ± 4.21	69.4 ± 3.60	50.9 ± 2.72	77.8 ± 3.58
12a		H	34.1 ± 2.36	46.1 ± 2.61	36.0 ± 1.94	27.1 ± 1.97
12b		CH_3	45.2 ± 2.92	25.9 ± 1.87	**20.0 ± 1.47**	39.9 ± 2.52
13			**14.4 ± 1.05**	**11.4 ± 0.98**	**15.6 ± 1.20**	**9.1 ± 1.10**
14			47.1 ± 3.14	57.1 ± 3.18	71.3 ± 3.65	45.6 ± 2.64
15			**10.8 ± 0.92**	**9.5 ± 0.84**	**13.7 ± 0.97**	**8.8 ± 0.93**
16			**9.0 ± 0.68**	**7.8 ± 0.42**	**12.2 ± 0.81**	**7.9 ± 0.68**
17			**17.4 ± 1.32**	**13.1 ± 1.10**	**16.3 ± 1.36**	**10.7 ± 1.23**

IC50 = IC50 (μg/ml): 1 - 10 (very strong). 11 - 20 (strong). 21 - 50 (moderate). 51 - 100 (weak) and above 100 (non-cytotoxic). CA-4 = combretastatin A-4.

shows wide spectrum cytotoxic activity with IC50 starting from 6.9 to 12.7 μM. Replacement of phenyl ring with acetyl group as in compound **5c** or unsubstituted indazole in compound **5a** and uncyclized chalcone as in compound **4a** exhibited a decrease within the activity against the four cell lines. Additionally, the cytotoxic activities of recent synthesized quinoline-3-carbonitrile **6-9**, compounds **6a** with 2-methoxy and **6c** with 2-propoxy group has broad spectrum activity virtually identical IC50, (22.6 ± 1.87 for HCT-116, 14.9 ± 1.23 for Hela, 17.7 ± 1.41 for PC3, 14.0 ± 1.41 for MCF-7), (19.8 ± 1.50 for HCT-116, 16.0 ± 1.32 for Hela, 18.9 ± 1.39 for PC3, 13.8 ± 1.42 for MCF-7) respectively. In comparison to compound **6c**, replacing propoxy group with ethoxy group in compound **6b** resulting in 1.5 times decrease in cytotoxic activity. Also replacing propoxy group with amino group in compound **7** resulting in reduce in activity

by almost 4 times. Compounds **8** and **9** shows low activity within the four cell lines. By replacing CH_2 of cyclohexanone chalcone **4a** by N-CH_3 chalcone **4b**, the cytotoxic activity is almost doubled against the four human cell lines. While the cytotoxic activity of **10a** and **11b** is ranged from moderate to weak activity but compounds **10b** and **11a** are inactive with IC50 > 100 μg/ml.

Also, the series of 4-substituted 1, 5-diphenyl pyrazole shows a range of intermediate to high cytotoxicity activity. Compounds with benzo chromene-3-carbonitrile substitution as in **15** and **16** results in very strong cytotoxic activity with IC50, (10.8 ± 0.92 for HCT-116, 9.5 ± 0.84 for Hela, 13.7 ± 0.97 for PC3, 8.8 ± 0.93 for MCF-7) and (9.0 ± 0.68 for HCT-116, 7.8 ± 0.42 for Hela, 7.9 ± 0.68 for PC3, 12.2 ± 0.81 for MCF-7) respectively. Replacement of aniline or substituted aniline in compounds **12a**, **b** by either 2-amino thiazole in compound **13** or substituted pyrazole in compound **17** enhance cytotoxic activity with IC50, (11.4 ± 0.98 for HCT-116, 14.4 ± 1.05 for Hela, 15.6 ± 1.20 for PC3, 9.1 ± 1.10 for MCF-7) and (17.4 ± 1.32 for HCT-116, 13.1 ± 1.10 for Hela, 16.3 ± 1.36 for PC3, 10.7 ± 1.23 for MCF-7) respectively.

3.2. Molecular Modeling

Molecular docking was performed for compounds that inhibited tubulin polymerization so as to explore attainable binding modes. The tubulin protein structure in complex with podophyllotoxin (PDB 1SA1) was used for the study [30] [31] [32]. In molecular docking, MOE programme (molecular operating environment) was used in our study. CA-4 is employed as lead compound due to its excellent affinity to colchicine binding site (**Figure 2**). Comparing to the lead molecule CA-4 and biological activity, we have a tendency to found that compounds **5b**, **15**, **16** and **17** have the lowest energy and give the most stable complexes with the receptor and are the most biological active against cell lines so we concluded that these new compounds have very good affinity binding with the protein. During this study, we observed that basic amino acid Lys (B254) is essential as it is bound with **15** *via* arene cation interaction and with **16** *via* hydrogen bond (angle NH…N = 101.9, distance = 2.9 A^0) (**Figure 4**, **Figure 5**). Also, it was found that Tyr (A224) is important for arene-arene interaction with **5b**, **15** and **17** (**Figure 3**, **Figure 4**, **Figure 6**). Also, Glu (A183) is bound to the amino group in **15** (**Figure 4**) *via* hydrogen bond (angle NH…O = 111.9, distance = 2.8 A^0). A Hydrogen bond is formed between a nitrogen atom in nitrile group in **17** (**Figure 6**) and hydroxyl side chain of ser (A140) (angle N…HO =1 02.9, distance = 2.7 A^0).

4. Experimental

Melting points (˚C) were recorded using a Fisher-Johns melting point apparatus and are uncorrected. Microanalyses (C, H, and N) were performed in Cairo University Microanalytical unit, and all the results within ±0.4. IR spectra (KBr) were recorded on Thermo Fisher Scientific Nicolet IS10. Spectrometer (ν in

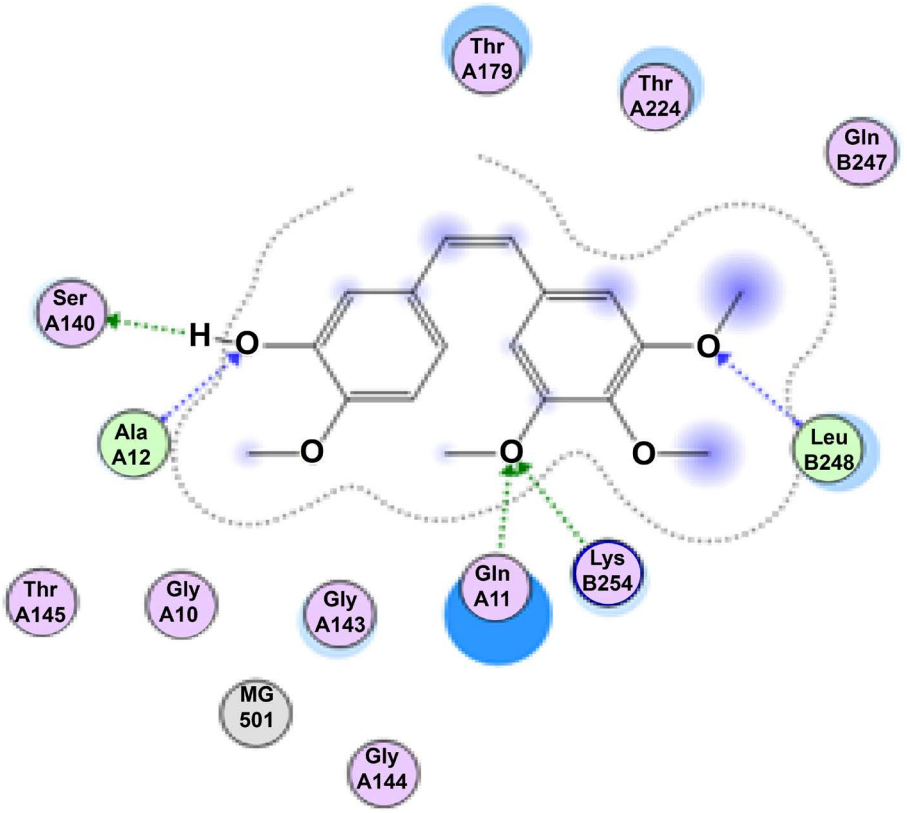


Figure 2. Binding of CA-4 with 1SA1 amino acids. (a) Gln: Glutamine; (b) Lys: Lysine; (c) Ala: Alanine; (d) Ser: Serine.

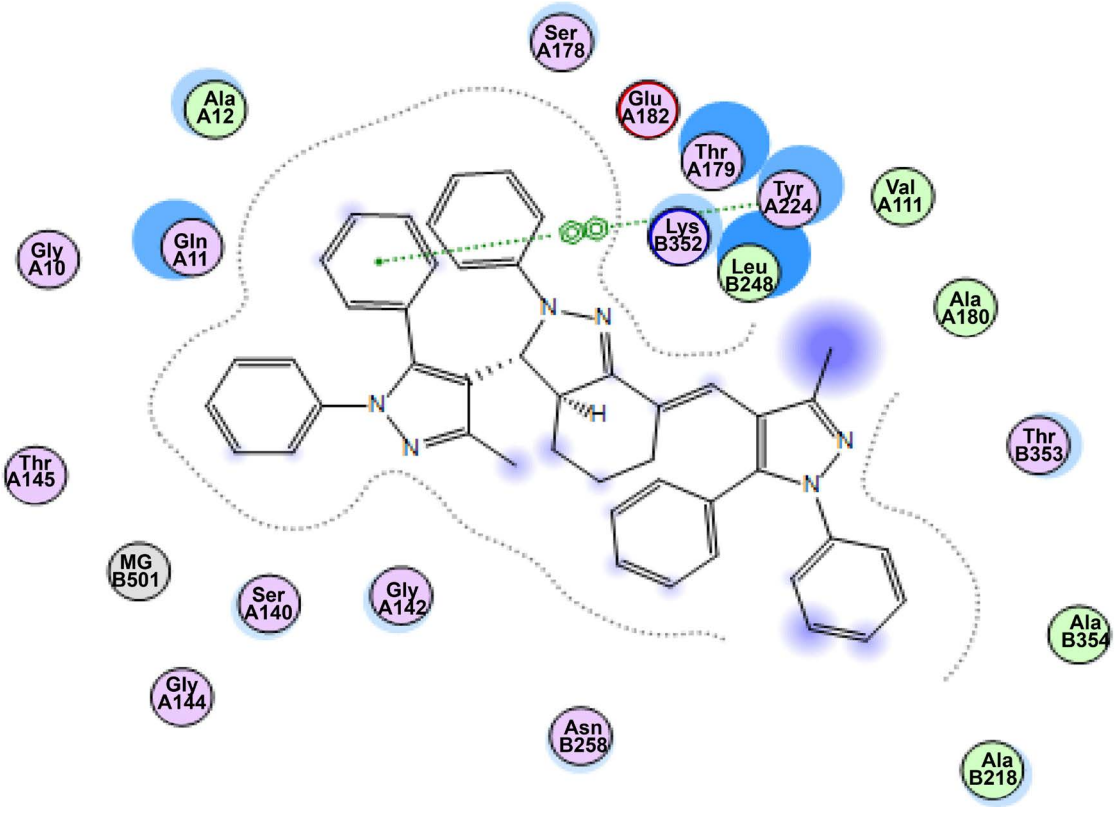


Figure 3. Binding of 5b with 1SA1 amino acids. (a) Tyr: Tyrosine.

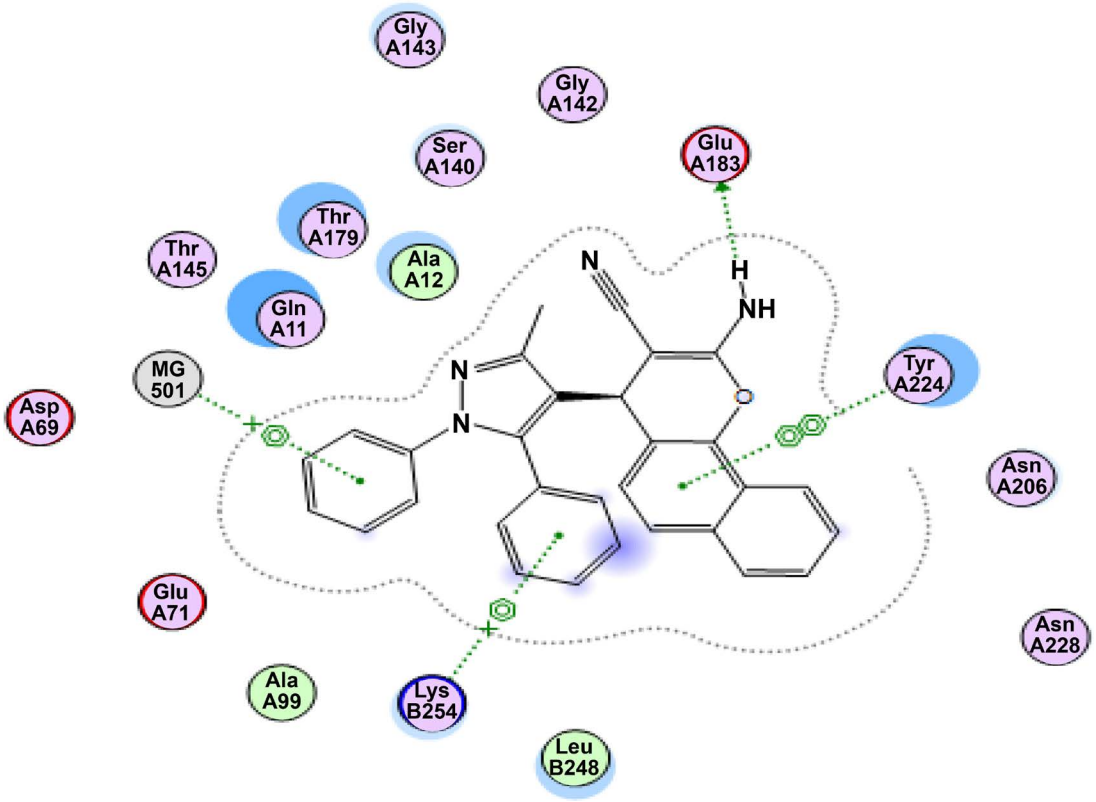


Figure 4. Binding of 15 with 1SA1 amino acids. (a) Lys: Lysine; (b) Tyr: Tyrosine; (c) Glu: Glutamic acid; (d) Mg^{+2} cation.

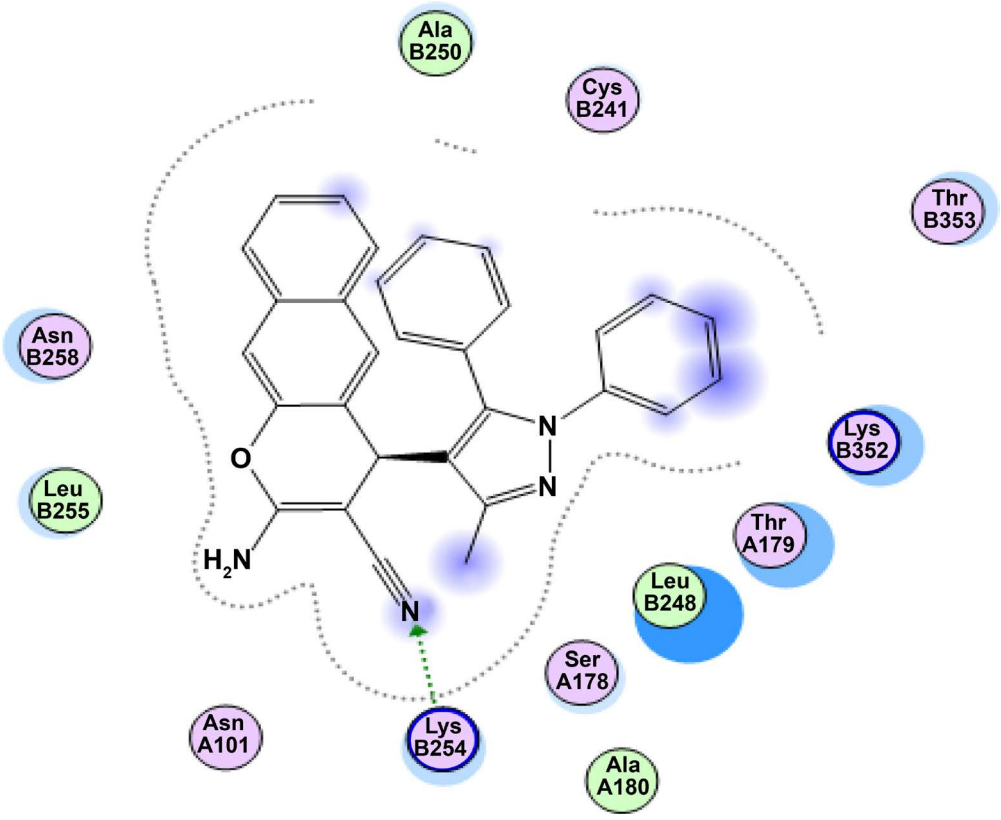


Figure 5. Binding of 16 with 1SA1 amino acids. (a) Lys: Lysine.

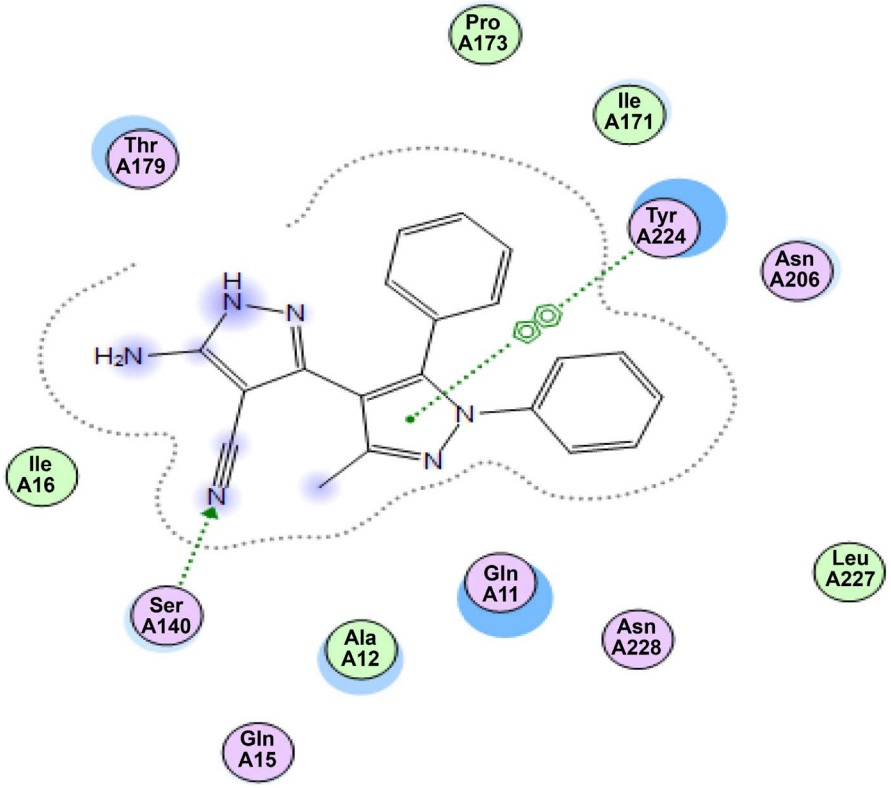


Figure 6. Binding of 17 with 1SA1 amino acids. (a) Tyr: Tyrosine; (b) Ser: Serine.

cm^{-1}) at Faculty of Pharmacy, Mansoura University. Mass MS (EI) *m/z* analyses were performed on Thermo Scientific DCQII, Faculty of Science, Mansoura University. ^{1}H and ^{13}C-NMR spectra were obtained in DMSO-d_6 or $CDCl_3$ on ASCEND spectrometer (400 MHz) Bruker at Georgia State University USA, Faculty of Pharmacy, Zagazig University and Faculty of Science, Kafr ElSheikh University. Reaction times were decided using TLC on Silica gel plates 60 F254 (E. Merk), and the spots were visualized by U.V (366, 245 nm). Compounds **2**, **3** were prepared as previously discussed in literature [16] [33].

4.1. General Method for Preparation of Chalcones 4a, b

Ethanolic solution (20 ml) of NaOH (1 g, 0.025 mol) was mixed with a solution of 3-methyl-1,5-diphenyl-1H– pyrazole-4-carbaldehyde (**3**) (1 g, 0.02 mol) and the appropriate cyclic ketones (0.01 mol) in ethyl alcohol (30 ml). The previous mixture was stirred at 25˚C for 2 h. The product obtained was then filtered, washed with water, dried and finally recrystallized from ethyl alcohol.

(2*E*,6*E*)-2,6-bis[(3-Methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)methylene]cyclohexanone (4a) as yellow solid; yield 45% (1 g); mp 230˚C - 233˚C; ^{1}H NMR (DMSO-d_6): δ 7.2 - 7.3 (m, 20H, Ar-H), 7.1 (d, J = 8.1 Hz, 2H, methylene-H), 2.5 (s, 6H, $2CH_3$), 2.1 (s, 4H, cyclohexanone-H), 1.6 (q, J = 7.1 Hz, 2H, cyclohexanone-H); ^{13}C NMR: δ 13 (2c), 25.7 (1c), 26.1 (2c) 115.7 (2c), 124.5 (4c), 126.2 (2c), 127.5 (4c), 128 (2c), 129.2 (4c), 129.3 (4c), 133 (2c), 139.8 (2c), 142.2 (2c), 143.8 (2c), 147.9 (2c), 150 (2c), 191 (1c); IR (KBr, cm^{-1}): 1670 (C=O), 1627

(C=N), 1547, 1489 (C=C); MS (EI) *m/z*: 586 (30%, M+); anal (calcd.) for $C_{40}H_{34}N_4$,C: 81.88, H: 5.84, N: 9.55. Found, C: 81.87, H: 5.85, N: 9.04.

(3*E*,5*E*)-1-Methyl-3,5-bis[(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)methylene]piperidin-4-one (4b) as brown solid; yield 75% (0.8 g); mp 300˚C - 302˚C; ^{1}H NMR (DMSO-d_6): 7.1 - 7.7 (m, 22H, Ar-H, 2H-methylene), 4.1 (s, 4H, CH_2NCH_2), 2.5 (s, 6H, $2CH_3$), 2.2 (s, 3H, N-CH_3); IR (KBr, cm^{-1}): 1670 (C=O); MS (EI) *m/z*: 601 (98.9%, M+); anal (calcd.) for $C_{40}H_{35}N_5$,C: 79.84, H: 5.86, N: 11.64. Found, C: 79.88, H: 5.85, N: 11.55.

4.2. General Procedure for Synthesis of 5a, c

Hydrazine hydrate (98%) (0.3 ml, 0.05 mol) introduced to a solution of **4a** in ethyl alcohol (99%) (20 ml) **5a** or ethanoic acid (10 ml) **5c** and the mixture was refluxed for 5 h. The solvent was decreased using evaporator. After cooling the separated product was filtered, dried and finally recrystallized from ethyl alcohol.

4.3. General Procedure for Synthesis of 5b

A mixture of chalcone **4a** (0.7 g, 0.005 mol), phenyl hydrazine (0.12 g, 0.005 mol) in glacial ethanoic acid (15 ml) was heated for 8 h. Then the mixture was diluted with ice-cold water. The precipitate was collected, washed with water, dried and purified by SiO_2 (preparative TLC in petroleum ether: ethyl acetate 3:1)

3-(3-Methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-7-(*E*)[(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)methylene]-3,3a,4,5,6,7-hexahydro-2*H*-indazole (5a): (1 g, 0.01 mol) of compound **4a** gave **5a** as white powder; yield 40% (0.4 g); mp 150˚C - 155˚C; ^{1}H NMR (DMSO-d_6): δ 8.1 (s, 1H, NH (D_2O exchangeable), 7.1 - 7.3 (m, 21H, 20 Ar-H, methylene-H), 2.5 (s, 3H, CH_3), 2.2 (t, J = 7.7 Hz, 2H, indazole H-4), 2.1 (s, 3H, CH_3), 1.9 (m, 4H, indazole-H_5, H_6); IR (KBr, cm^{-1}): absence of C=O at 1670, 3410 (NH); MS (EI) *m/z*: 600 (8.8%, M+); anal (calcd.) for $C_{40}H_{34}N_6$, C: 79.97, H: 6.04, N: 13.99. Found, C: 79.85, H: 6.01, N: 13.78.

3-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-7-(*E*)[(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)methylene]-2-phenyl-3,3a,4,5,6,7-hexahydro-2*H*-indazole (5b): as yellow solid; yield 83% (0.5 g); mp 129˚C - 130˚C. ^{1}H NMR (DMSO-d_6): δ 7.4 - 7.7 (m, 26H, Ar-H, methylene-H), 2.7 (t, J = 7.3 Hz, 2H, indazole-H4), 2.4 (s, 3H, CH_3-pyrazole), 2.1 (s, 3H, CH_3-pyrazole), 1.9 (m, J = 7.1 Hz, 4H, indazole –H5, H6); IR (KBr, cm^{-1}): 1598 (C=C), absence of C=O at 1670; MS (EI) *m/z*: 676 (31%, M+); anal calcd for $C_{46}H_{38}N_6$: C: 81.63, H: 5.96, N: 12.42. Found: C, 81.53, H: 5.87 N: 12.38.

1-{[3-(3-Methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-7-(*E*)[(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)methylene]-3,3a,4,5,6,7-hexahydro-2*H*-indazol-2-yl}ethanone (5c): (0.7 g, 0.005 mol) of compound **4a** gave **5c** as buff solid; yield 57% (0.4 g); mp 138˚C - 140˚C; ^{1}H NMR (DMSO-d_6): δ 7.1 - 7.3 (m, 21H, 20 Ar-H, methylene-H), 2.7 (t, J = 7.7 Hz, 2H, indazole H-4), 2.2 (s, 3H, $COCH_3$), 2.5 (s,

3H, CH_3-pyrazole), 2.1 (s, 3H, CH_3-pyrazole), 1.9 (m, 4H, indazole-H_5, H_6); IR (KBr, cm^{-1}): 1656 (C=O), 1504 (C=C); MS (EI) *m/z*: 642 (59%, M+); anal (calcd.) for $C_{42}H_{36}N_6$, C: 78.33, H: 5.85, N: 13.99. Found, C: 78.5, H: 5.01, N: 13.78.

4.4. Preparation of 6a-c

A mixture of compound **4a** (0.6 g, 1 mmol) and malononitrile (0.07 g, 1 mmol) was added to freshly prepared solution of sodium alkoxide (15 ml), (0.3 g, 0.014 mol) of sodium in 100 ml of the appropriate alcohol. The mixture kept at room temperature for the ideal time. The triggered product became collected by filtration, washed with ethanol and recrystallized from ethanol/ DMF.

2-Methoxy-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-8-(*E*)[(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)methylene]-5,6,7,8-tetrahydroquinoline-3-carbonitrile (6a) as white solid; yield 66% (0.4 g); m.p 160˚C - 163˚C; ^{1}H NMR (DMSO-d_6): δ 8.1 (s, 1H, methylene-H), 7.4 - 7.7 (m, 20H, 20Ar-H), 4.1 (s, 3H, OCH_3), 3.1 (t, J = 7.5 Hz, 2H, quinoline-H5),, 2.5 (s, 6H, $2CH_3$), 2.1 (m, 4H, quinoline-H6, H7); ^{13}C NMR: δ 11.6 (1c), 12.5 (1c), 21.5 (1c), 26.7 (1c), 32.2 (1c), 54.1 (1c), 111.7 (1c), 112.2 (1c), 116.3 (1c), 119.7 (1c), 120.8 (1c), 123.9 (2c), 124.8 (2c), 126.5 (1c), 127 (1c), 128.2 (1c), 128.4 (2c), 128.51 (4c), 128.6 (4c), 129.3 (2c), 129.8 (2c), 129.9 (2c), 130.3 (1c), 131 (1c), 139.4 (1c), 139.6 (1c), 139.9 (1c), 140.5 (1c), 141 (1c), 147.2 (1c), 147.4 (1c), 159.5 (1c); IR (KBr, cm^{-1}): absence of CO peak at 1670, 2221 (CN), 1598 (CN ring),; MS (EI) *m/z*: 664.9 (69%, M+); anal. calcd for $C_{44}H_{36}N_6$: C: 79.49, H: 5.46, N: 12.64. Found: C: 79.2, H: 5.41, N: 12.64.

2-Ethoxy-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-8-(*E*)[(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)methylene]-5,6,7,8-tetrahydroquinoline-3-carbonitrile (6b) as buff solid; yield 50% (0.3 g); mp 130˚C - 135˚C; ^{1}H NMR (DMSO-d_6): δ 6.5 - 7.3 (m, 21H, 20Ar-H, H-methylene), 3.8 (m, 2H, OCH_2), 3.1 (t, 2H, J = 7.1 Hz, quinolineH-5), 2.5 (s, 6H, $2CH_3$), 2.1 (m, 4H, quinoline-H6, H7), 1.6 (t, J = 7.5 Hz, 3H, CH_3); IR (KBr, cm^{-1}): absence of CO, 2220 (CN); MS (EI) *m/z*: 678 (12%, M+); anal. calcd for $C_{45}H_{38}N_6$: C: 79.62, H: 5.64, N: 12.38. Found: C: 79.21, H: 5.43, N: 12.04.

4-(3-Methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-8-(*E*)[(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)methylene]-2-propoxy-5,6,7,8-tetrahydroquinoline-3-carbonitrile (6c) as white solid; yield 71% (0.5 g); mp 141˚C -142.5˚C; ^{1}H NMR (DMSO-d_6): δ 8.1 (s, 1H, H-methylene), 7.5 -7.8 (m, 20H, 20Ar-H), 3.8 (t, J = 7.8 Hz, 2H, OCH_2), 3.1 (t, 2H, J = 7.1 Hz, quinoline-H5), 2.5 (s, 6H, $2CH_3$), 1.9 (m, 4H, quinolineH6, H7), 1.8 (m, 2H, C-CH_2), 1.6 (m, 3H,CH_3); ^{13}CNMR: δ 11.6 (2c), 12.5 (2c), 21.5 (1c), 26.7 (1c), 32.3 (1c), 54.1 (1c), 111.7 (1c), 112.2 (1c), 116.3 (1c), 119.7 (1c), 120.8 (1c), 123.9 (2c), 124.8 (2c), 126.5 (1c), 127 (1c), 128.2 (1c), 128.4 (2c), 128.51 (2c), 128.59 (2c), 128.6 (2c), 128.7 (2c), 129.3 (2c), 129.8 (2c), 129.9 (2c), 130.3 (1c), 131 (1c), 139.4 (1c), 139.6 (1c), 139.9 (1c), 140.5 (1c), 141 (1c), 147.2 (1c), 147.4 (1c), 159.5 (1c); IR (KBr, cm^{-1}): absence of CO, 2221 (CN), 1598 (CN ring); MS (EI) *m/z*: 692 (20%, M+); anal. calcd for $C_{46}H_{40}N_6$: C: 79.74, H: 5.82, N: 12.13. Found: C: 79.03, H: 5.31, N: 12.11.

4.5. Preparation of 2-Amino-4-(3-Methyl-1,5-Diphenyl-1*H*-Pyrazol-4-yl)-8-(*E*) [(3-Methyl-1,5-Diphenyl-1*H*-Pyrazol-4-yl) Methylene] -5,6,7,8-Tetrahydroquinoline-3-Carbonitrile (7)

Compound **4a** (2.6 g, 0.005 mol) was mixed with malononitrile (0.3 g, 0.005 mol) and ammonium acetate (2.7 g, 0.04 mol) in ethyl alcohol (99%) (30 ml).The mixture was refluxed for 7 h, and then concentrated under reduced pressure. After cooling, the precipitated product was filtered, dried and purified by SiO_2 (preparative TLC petroleum ether: ethyl acetate 3:1) to yield compound **7** as yellow powder; yield 71% (2 g); mp 300˚C - 302˚C; ^{1}H NMR (DMSO-d_6): δ 7.1 - 7.3 (m, 21H, Ar-H, H-methylene), 6.7 (s, 2H, NH_2 (D2O exchangeable), 3.1 (t, 2H, J = 7.1 Hz, quinoline-H5), 2.5 (s, 3H, CH_3), 2.1 (s, 3H, CH_3), 1.9 (m, 4H, quinolineH6, H7); IR (KBr, cm^{-1}): 2215 (CN), 3413 (NH_2), 1598 (C=N ring); MS (EI) *m/z*: 649.3 (20.3%, M+); anal. calcd for $C_{43}H_{35}N_7$: C: 79.33, H: 5.4, N: 15.4. Found: 79.46, H: 5.4, N: 15.09.

4.6. Preparation of 4-(3-Methyl-1,5-Diphenyl-1*H*-Pyrazol-4-yl)-8-(*E*) [(3-Methyl-1,5-Diphenyl-1*H*-Pyrazol-4-yl) Methylene]-2-Oxo-1,2,5,6,7,8-Hexahydroquinoline-3-Carbonitrile (8)

Compound **4a** (1.5 g, 0.01 mol) was mixed with ethylcyanoacetate (0.3 g, 0.1 mol) and ammonium acetate (2 g, 0.1 mol) in ethyl alcohol (99%) (50 ml), the reaction mixture was refluxed for 15 h. After cooling, the formed precipitate was filtered, washed with ethyl alcohol, then washed with water, dried and purified by SiO_2 (preparative TLC petroleum ether: ethylacetate 3:1) to yield compound **8** as yellow powder; yield 41% (0.7 g); mp 152˚C - 153˚C. ^{1}H NMR (DMSO-d_6): δ 9.1 (s, 1H, NH (D_2O exchangeable), 7.1 - 7.7 (m, 20H, Ar-H), 6.3 (s, 1H, H-methylene), 3.3 (m, 4H, quinoline-H5, H7), 2.1 (s, 6H, 2CH_3), 1.9 (m, 2H, quinoline-H6); IR (KBr, cm^{-1}): 2221 (CN), 3400 (NH); MS (EI) *m/z*: 650 (57%, M+); anal. calcd for $C_{43}H_{34}N_7$: C: 79.48, H: 5.4, N: 12.78. Found: C: 79.33, H: 5.4, N: 12.91.

4.7. Preparation of 2-Chloro-4-(3-Methyl-1,5-Diphenyl-1*H*-Pyrazol-4-yl)-8-(*E*)[(3 -Methyl-1,5-Diphenyl-1*H*-Pyrazol-4-yl) Methylene]- 1,2,5,6,7,8-Hexahydroquinoline-3-Carbonitrile (9)

A mixture of **8** (0.7 g, 0.0013 mol), phosphorus oxychloride (2.5 ml, 0.026 mol) and *N,N*-diethylaniline (DEA) (0.1 ml) was heated for 6 h. After cooling by ice (15 ml), the product turned into filteration, dried and washed with ice-water to yield compound **9** as yellow powder; yield 71% (0.5 g); mp 190˚C - 192˚C; ^{1}H NMR (DMSO-d_6): δ 7.1 - 7.7 (m, 20H, Ar-H), 6.1 (s, 1H, H-methylene), 3.3 (m, 4H, quinoline-H5, H7), 2.1 (s, 6H, 2CH_3), 1.9 (m, 2H, quinoline-H6). IR (KBr, cm^{-1}): 2220 (CN), absence of C=O band at 1670; MS (EI) *m/z*: 673 (11%, M++2), 671 (20%, M+); anal. calcd for $C_{43}H_{33}ClN_6$: C: 76.85, H: 5.85, Cl: 5.13, N: 12.46.

Found: C: 76.2, H: 5.11, Cl: 5.5, N: 12.2.

4.8. Preparation of 10a, b

The *α*, *β*-unsaturated ketone **4a** or **4b** (0.01 mol) was mixed with thiourea (0.8 g, 0.01 mol) and Sodium metal (0.5 g) in butyl alcohol (50 mL). The mixture was refluxed for 10 h. The extra of solvent became concentrated in *vacuo* and the residue changed into pH 6. The separated stable turned into filteration, washed with water, dried and purified through SiO_2 (preparative TLC petroleum ether: ethyl acetate 3:1).

4-(3-Methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-8-(*E*)[(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)methylene]-4,4a,5,6,7,8-hexahydroquinazoline-2(3*H*)-thione (10a): As yellow solid; yield 20% (0.3 g); mp 138˚C - 140˚C; ^{1}H NMR (DMSO-d_6): *δ* 8.3 (s, 1H, SH), 7.1 - 7.3 (m, 20H, 20Ar-H), 5.4 (s, 1H, H-methylene), 2.8 (m, 4H, quinazoline-H5, H7), 2.4 (s, 6H, 2 CH_3), 1.6 (m, 2H, quinazoline-H6); MS (EI) *m/z*: 644 (51%, M+); anal. calcd for $C_{41}H_{34}N_6S$: C: 76.37, H: 5.63, N: 13.03, S: 4.97. Found: C: 76.2, H: 5.11, N: 13.2, S: 4.5.

6-Methyl-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-8-(*E*)[(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)methylene]-4,4a,5,6,7,8-hexahydropyrido[4,3-*d*]pyrimidine-2(3*H*)-thione (10b): As brown solid; yield 15% (0.2 g); mp 150˚C - 153˚C; ^{1}H NMR (DMSO-d_6): *δ* 9.2 (d, 1H, SH), 7.1 - 7.3 (m, 20H, 20Ar-H), 4.8 (s, 1H, H-methylene), 3.3 (s, 4H, pyridine-H2,H6), 2.4 (s, 6H, 2CH_3), 2.2 (s, 3H, N-CH_3); MS (EI) *m/z*: 659.1 (5.5%, M+); anal. calcd for $C_{41}H_{35}N_7S$: C: 74.63, H: 5.65, N: 14.86; S: 4.86. Found: C: 74.2, H: 5.55, N: 14.2, S: 4.67.

4.9. Preparation of 11a, b

A solution of 2-aminothiazole (0.08 g, 0.01 mol) and the *α*,*β*-unsaturated ketone **4a** or **4b** (0.5 g, 0.01 mol) in glacial ethanoic acid (20 ml) was refluxed for 20 h. Solvent was dried under reduced pressure; then washed with water. The obtained product was recrystallized from ethyl alcohol.

5-(3-Methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-9-(*E*)[(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)methylene]-6,7,8,9-tetrahydro-5*H*-thiazolo[2,3-*b*]quinazoline (11a): As yellow crystals; yield 80% (0.4 g); mp 200˚C - 201˚C; ^{1}H NMR (DMSO-d_6): *δ* 7.1 - 7.3 (m, 22H, 20Ar-H and 2Thiazole-H), 6.3 (s, 1H, H-methylene), 4.5 (s, 1H, thiazolo quinazoline-H5), 2.8 (m, 2H, thiazolo quinazoline-H8), 2.5 (s, 6H, 2 CH_3), 1.6 (m, 4H, thiazolo quinazoline-H6, H7). ^{13}CNMR: *δ* 13 (1c), 14 (1c), 23.3 (1c), 23.2 (1c), 25 (1c), 56 (1c), 97 (1c), 113 (1c), 119 (1c), 124 (1c), 124.5 (4c), 126.4 (2c), 127.5 (4c), 128.8 (2c), 129.9 (8c), 132 (2c), 133 (3c), 134.1 (1c), 139.2 (2c), 142 (1c), 146 (1c), 148.7 (1c), 150 (1c), 152.9 (1c), 158.4 (1c); MS (EI) *m/z*: 668 (13%, M+); anal. calcd for $C_{43}H_{36}N_6S$: C: 77.22, H: 5.43, N: 12.56, S: 4.79. Found: C: 77.20, H: 5.81, N: 12.40, S: 4.91.

7-Methyl-5-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-9-(*E*)[(3-methyl-1,5-diphenyl-1*H*-pyzol-4-yl)methylene]-6,7,8,9-tetrahydro-5*H*-pyrido[4,3-*d*]t

hiazolo[3,2-*a*]pyrimidine (11b): As brown crystals; yield 80% (0.4 g); mp 130˚C - 133˚C; ^{1}H NMR (DMSO-d_6): δ 7.1 - 7.3 (m, 22H, 20Ar-H and 2Thiazole-H), 6.3 (s, 1H, H-methylene), 4.5 (s, 1H, pyrimidine-H4), 3.1 (m, 4H, pyridine H2, H6), 2.4 (s, 6H, CH_3), 2.2 (s, 3H, N-CH_3); IR (KBr, cm^{-1}): 1529 (C=N ring); MS (EI) *m/z*: 683.1 (5%, M+; anal. calcd for $C_{43}H_{37}N_7S$: C: 75.52, H: 5.45, N: 14.34, S: 4.69. Found: C: 75.22, H: 5.82, N :14.40, S: 4.95.

4.10. Preparation of 12a, b and 13

A mixture of compound **3** (2 g, 0.1 mol) and appropriate aromatic amine in glacial ethanoic acid (20 ml) was heated under reflux for 5 h. The solid was precipitated by adding cold water. The separated solid turned into filteration, washed with water, dried and purified by means of SiO_2 (preparative TLC petroleum ether: ethyl acetate 3:1).

[1-(3-Methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)]-*N*-phenylmethanimine (12a): aniline (0.8 g, 0.1 mol) to yield pure white powder; yield 38% (1 g); mp 120˚C - 122˚C; ^{1}H NMR (DMSO-d_6): δ 8.1 (s, 1H, CH=N), 7.2 - 7.4 (m, 15H, Ar-H), 2.6 (s, 3H, CH_3 –pyrazole); ^{13}C NMR: δ 14.3 (1c), 116.9 (1c), 120.5 (2c), 125 (2c), 125.2 (1c), 127.6 (1c), 128.1 (1c), 128.7 (2c), 128.9 (2c), 129.1 (2c), 129.2 (2c), 130.2 (1c), 138.8 (1c), 145.7 (1c), 148.7 (1c), 152.7 (1c), 153.5 (1c); MS (EI) *m/z*: 337.1 (40.4%, M+); anal. calcd for $C_{23}H_{19}N_3$: C: 81.87; H, 5.68; N, 12.45. Found: C: 81.45, H: 5.81, N: 12.74.

[1-(3-Methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-*N*-(3-methylphenyl)]methanimine (12b): *m*-toluidine (0.7 g, 0.1 mol) to yield brown powder; yield 40%(1 g); mp 120˚C - 122˚C; ^{1}H NMR (DMSO-d_6): δ 8.1(s, 1H, CH=N), 7.2 - 7.4 (m, 14H, Ar-H), 2.4 (s, 3H, CH_3 –pyrazole), 2.2 (s, 3H, CH_3); MS (EI) *m/z*: 351.1 (38.7%, M+); anal. calcd for $C_{24}H_{21}N_3$: C: 82.02; H:6.02; N: 11.96. Found: C: 82.45, H: 6.81, N: 11.74.

[1-(3-Methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)-*N*-(thiazol-2-yl)]methanimine (13): 2-aminothiazole (0.7 g, 0.1 mol) to yield buff powder; 62% (1.5 g); mp 130˚C - 131.5˚C; ^{1}H NMR (DMSO-d_6): δ 8.1 (s, 1H, CH=N), 7.1 - 7.8 (m, 12H, Ar-H), 2.5 (s, 3H, CH_3 –pyrazole). anal. calcd for $C_{20}H_{16}N_4S$: C: 69.74; H: 4.68; N: 16.27, S: 9.31. Found: C: 69.43, H: 4.81, N: 16.74, S: 9.22.

4.11. Preparation of [2-(3-Methyl-1,5-Diphenyl-1*H*-Pyrazol-4-yl) Methylene] Malononitrile (14)

Mixture of methyl-1, 5-diphenyl-1H-pyrazole-4-carbaldehyde **3** (3.5 g, 1 mol), malononitrile (0.9 g, 1 mol), in ethyl alcohol 95% (20 ml) were refluxed for 12 h. After of entirety of the reaction with the aid of TLC, the mixture changed into cooling to 25˚C, filtered and washed with water. The resulting solid product was recrystallized from methanol to yield compound **14** as pure yellow crystals 75% (3 g); mp 135˚C - 136˚C; ^{1}H NMR (DMSO-d_6): δ 8.1 (s, 1H, ethylenic-H), 7.4 (d, 3H, J = 5 Hz, Ar-H), 7.3 (s, 3H, Ar-H), 7.2 (d, 4H, J = 5 Hz, Ar-H), 2.5 (d, 3H, J = 5 Hz, CH_3).^{13}C NMR: δ 14.2 (1c), 80.7 (1c), 113.3 (1c), 114.2 (1c), 114.6 (1c),

125.3 (2c), 127.5 (1c), 128.5 (1c), 129 (1c), 129.1(1c), 129.8 (1c), 129.9 (2c),138.3 (2c), 146.1 (1c), 149.1 (2c), 154.3 (1c); IR (KBr, cm^{-1}): 2221 (CN), 1597 (C=C, C=N of ring); MS (EI) *m/z*: 310.1 (100%, M+); anal. calcd for $C_{20}H_{14}N_4$: C: 77.40; H: 4.55; N: 18.05. Found: C: 77.43, H: 4.81, N: 18.43.

4.12. Preparation of 15, 16

Mixture of compound **14** (3.5 g, 1 mmol), the appropriate naphthol (1 mmol) and methanesulfonic acid (1.2 g, 1 mmol) in acetonitrile (5 ml) were refluxed for 5 h. After completion of the reaction, the aggregate turned into cooling to room temperature and filtered. The filtrate wad washed by 5% $NaHCO_3$ and dried over $MgSO_4$. The solvent turned into evaporated. The resulting solid product turned into recrystallization from methyl alcohol.

[2-Amino-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)]-4*H*-benzo[*h*]chromene-3-carbonitrile (15): Pure white crystals; yield 50% (3 g); mp 120˚C - 121˚C. ^{1}H NMR (DMSO-d_6): δ 7.2 - 7.4 (m, 16H, Ar-H), 6.9 (s, 2H, NH_2 (D_2O exchangeable), 4.7 (s, 1H, benzochromene-H4), 1.7 (s, 3H, CH_3); IR (KBr, cm^{-1}): 2188 (CN), 3448 (NH_2); anal.cald for $C_{30}H_{22}N_4$: C: 79.27; H: s4.88; N: 12.33. Found: C: 79.6; H: 4.5; N: 12.8.

[2-Amino-4-(3-methyl-1,5-diphenyl-1*H*-pyrazol-4-yl)]-4*H*-benzo[*g*]chromene-3-carbonitrile (16): To give pure white crystals; yield 40% (2 g); mp 150˚C - 151˚C. ^{1}H NMR (DMSO-d_6): δ 9.7 (s, 2H, NH_2 (D_2O exchangeable), 7.1 - 7.7 (m, 16H, Ar-H), 3.3 (s, 1H, H-4benzochromene), 2.5 (s, 3H, CH_3); IR (KBr, cm^{-1}): 2188 (CN), 3448 (NH_2); MS (EI) *m/z*: 454.3 (10.5%, M+); anal. calcd for $C_{30}H_{22}N_4$: C: 79.27, H: 4.88, N: 12.33. Found: C: 79.43, H: 4.81, N: 12.74.

4.13. Preparation of 17

A solution of compound **16** (4.6 g, 2 mol) in15 ml of ethanol was added to 80% hydrazine hydrate (0.6 ml, 1 mol). The mixture was stirred at 25˚C for 4 hrs, the solvent was evaporated under reduced pressure. The residue was washed with ethyl alcohol, recrystallized from the water.

[5-Amino-3'-methyl-1',5'-diphenyl-1*H*,1'*H*-3,4'-bipyrazole]-4-carbonitrile (17): As white needles; yield 40% (1 g); mp 197˚C - 198˚C; ^{1}H NMR (DMSO-d_6): δ 7.5 (s, 1H, NH (D_2O exchangeable), 7.1 - 7.4 (m, 10H, Ar-H), 6.4 (s, 2H, NH_2(D_2O exchangeable), 2.5 (d, 3H, J = 5 Hz, CH_3); IR (KBr, cm^{-1}): 2200 (CN), 3420 (NH_2); MS (EI) *m/z*: 340 (13.3%, M+); anal. calcd for $C_{20}H_{16}N_6$: C: 70.57, H: 4.74, N: 24.69. Found: C: 70.43, H: 4.25, N: 24.74.

5. Conclusion

A series of new compounds have been synthesized using efficient methods and tested for their biological activities. There is a strong correlation between molecular modeling and biological screening results which suppose that the structural modification of the lead structure affects the activity in a predictable manner; as shown in (**Figures 2-6**), CA-4 shows highest binding affinity with its methoxy

phenyl moiety and 2C bridge. Compound **5b** with 6C bridge and the methoxy phenyl moiety is replaced by diphenyl pyrazole ring gives good binding with colchicine binding site. Compounds **15** and **16** with a 4C spacer as double bond is replaced by chromene, ring A is replaced by diphenyl pyrazole moiety and ring B is replaced by naphthalene, have a good binding affinity with the protein but compound **15** have the best binding affinity. In compound **17**, after removing 2C distance, replacing ring A and B by substituted pyrazoles, we found that the activity as tubulin inhibitor highly increased and it's been considered as strong colchicine binding site agent.

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