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Construction, molecular docking, antimicrobial and antioxidant activity of some novel 3-substitued indole derivatives using 3-Acetyl indole

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Abstract:

This dissertation presents a method for the synthesis of substituted indoles bearing heterocyclic substituents onto the 3- position. The route for the synthesis of the heterocyclic substituents indoles starts from 3-acetyl indole nucleus depend on two parts. **One part:** Reaction of 3-acetylindole **1** with hydrazide compounds such as phenyl hydrazine, hydrazine hydrate, and thio-semicarbazide, to yield 3-(1-(2-phenylhydrazono) ethyl)-*1H*-indole **2**, 3-(1-hydrazonoethyl)-*1H*-indole **6**, and thiosemicarbazone **10** respectively. 3-(1-Hydrazonoethyl)-*1H*-indole **6** reacted with thiophene-2-carboxaldeyde, isatin and 3-acetyl indole. In the same way, 3-(1-(2-phenylhydrazono) ethyl)-*1H*-indole **2** reacted with thioglycollic acid, glycine and benzaldehyde. 3-Acetylindole **1** thiosemicarbazone reacted with acetic anhydride, piperidine, concentration of hydrochloric acid and thiophene-2-carboxaldeyde. **Second part.** Reaction of 3-acetyl indole **1** with amines compounds such as *p*-nitroaniline to formed Schiff base **15** which it reacted with thioglycollic acid to give compound **16**. 3-Acetyl indole **1** reacted with ethylene diamine to afford bis imine indole to afford compound **17**. The reports of this docking study revealed that the new compounds exhibit

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good antibacterial activity. The synthesized compounds screened in *vitro* for their antibacterial activity revealed remarkable inhibitory effects against the selected microorganisms. And also an anti-oxidant activity studying for some synthesized compounds. Structures of the newly synthesized compounds examination by spectral data (IR, ^{1}H- NMR and ^{13}C -NMR).

Keywords: 3-Acetyl indole; Biological Evaluations; Molecular Docking; thioglycollic acid; ethylene diamine and hydrazine hydrate.

Introduction:

Indole is considered a heterocyclic aromatic organic scaffold that consists of a five- membered pyrrole ring fused to a-six membered benzene compound through position 2- and 3- of pyrrole ring. It assembles a bicyclic structure and incorporates a heterocyclic system containing 10 electrons from (four double bonds and therefore the lone pair from the nitrogen atom), which makes them aromatic in nature. Another name of indole, is also called benzopyrrole, it is available in a number of flower oils, such as jasmine and orange blossom, and founded in coal tar, and also in fecal matter [1, 2]. 3-Acetylindole derivatives have been in the center of attention of researchers over many years due to the high practical value of these compounds, in the first place, the unusually broad spectrum of biological activities. Such as, 4-(*1H*-indol-3-yl)-2-hydroxy-4-oxobut-2-enoic acid **A** was used as anti-HIV agent [3], other compounds derived from 3-acetylindoles were effective in causing a marked increase in growth inhibition activity against various types of bacteria such as compound 5-((4-(*1H*-Indol-3-yl) thiazol-2-yl) diazenyl)-3-cyano-4-methyl-2-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl) thio)-6- phenylpyridin-2-yl) **B,** methyl 2-(2-(1H-indol-3-yl)-2-methyl-7-phenyl-5-thioxo-5,6-dihydrothiazolo[4,5-d]pyrimidin-3(*2H*)-yl)benzoate **C** [4-5], fungi such as **D** [6] (Figure).

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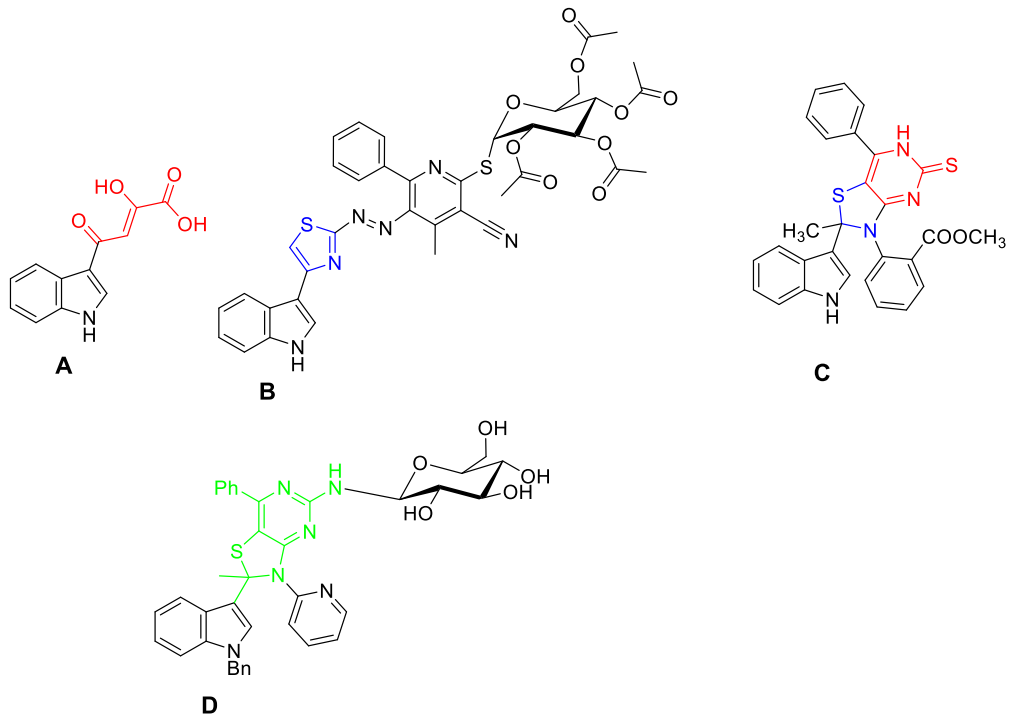

Figure 1. Some compounds of 3-substited indole appearance biological activity.

Results and Discussion

Chemistry

In previous work, the heterocyclic compounds studied appeared to have a wide range of medical and biological applications and are important for human life. In this communication, we are here to discuss the preparation of new sequence of heterocyclic compounds derived from 3-acetylindole **1**.

1-Reaction of 3- acetyl indole with hydrazines compounds

a) Condensation of 3-acetylindole with phenyl hydrazine

The reactivity of 3-acetylindole **1** was studies toward some hydrazine's derivatives. Thus, phenyl hydrazine was reacted as nitrogen bases with 3-acetylindole **1**, in the first reaction step to synthesized hydrazone (Schiff base or imine) **2** [7] scheme **1**.

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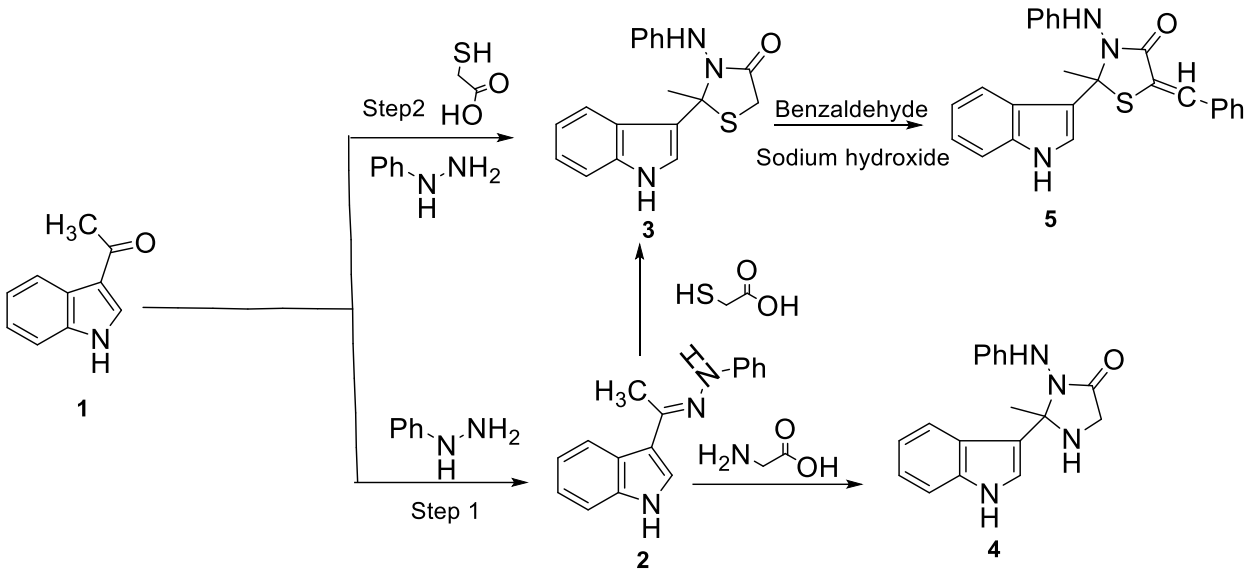

Scheme **1**. Synthesized compounds **2-5**

The Mechanism of Schiff base synthesis as shown in (figure 1). *First step*, nucleophilic addition of phenylhydrazine to the carbonyl group of 3-acetyl indole to forming unstable carbinolamine intermediates through transferring the proton to the oxygen from nitrogen. *Second step*, Protonation of the carbinolamine oxygen through the use of an acid catalyst such as (*p*-toluen sulfonic acid) then, converts the (–OH) into a good leaving group (H_2O), and removed the molecule of water to produce an iminium ion. Loss of a proton from nitrogen produce Schiff Bases (imines) **2**.

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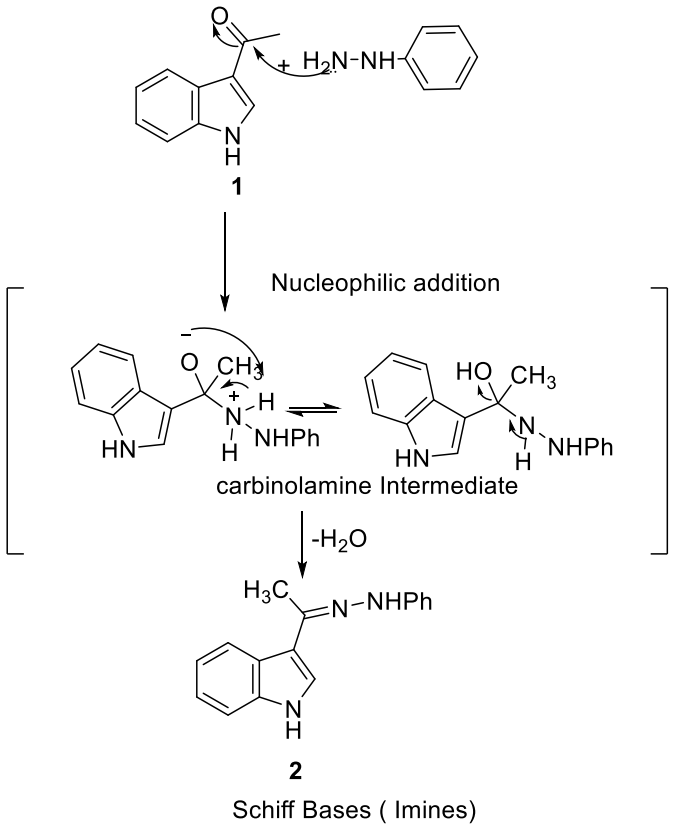

Figure 2. Formation of Schiff Bases (imines) **2**

Schiff base compound **2** was identified by melting point and R_f values, which underwent cyclo addition with thioglycolic acid by the attack of the sulfur nucleophile on the imine carbon. The last step involves intramolecular cyclization with the elimination molecule of water to produce thiazolidin-4-ones. **3** figure 2, this reaction proceed in acidic medium and compound **3** was confirmed by ^{1}H-NMR spectrum was showed a signal as the singlet at 11.29 ppm represented to (NHPh) and another signal at 3.59 ppm as the singlet due to methylene group of thiazolidinone ring. ^{13}C-NMR of compound **3** was appeared a peak at 176.96 ppm established to carbon atom (C=O) of thiazolidinone ring and another carbon at 40.31 ppm of methylene group.

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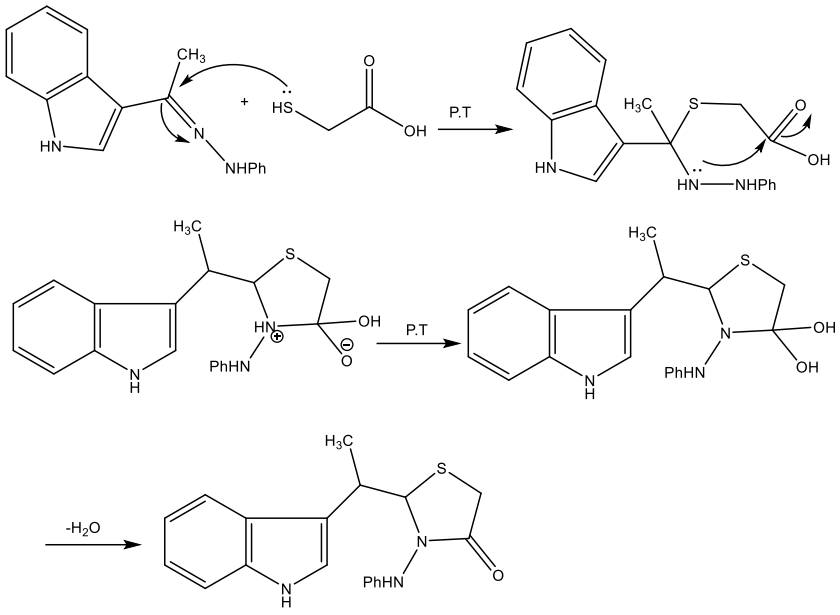

Figure 3. Formation of thiazolidinone derivatives **3**

In addition, the compound of Schiff base **2** was refluxed with glycine in toluene as the solvent to get imidazolidin-4-one derivatives **4** scheme1. Furthermore, IR spectrum of 2-(*1H*-indol-3-yl)-2-methyl-3-(phenylamino) imidazolidin-4-one **4** revealed the appearance of absorption bands at 3142 and 1672 cm^{-1} represented of NH and C=O groups respectively and disappearance (C=N) at 1610 cm^{-1} Also, ^{1}H-NMR spectra of compound **4** elucidation a signal as a doublet at 3.35-3.37 ppm due to methylene group (-CH_2-) and also found a signal as singlet at 7.63 ppm corresponding to NH of imidazoline. The proposed mechanism of this reaction in figure 4.

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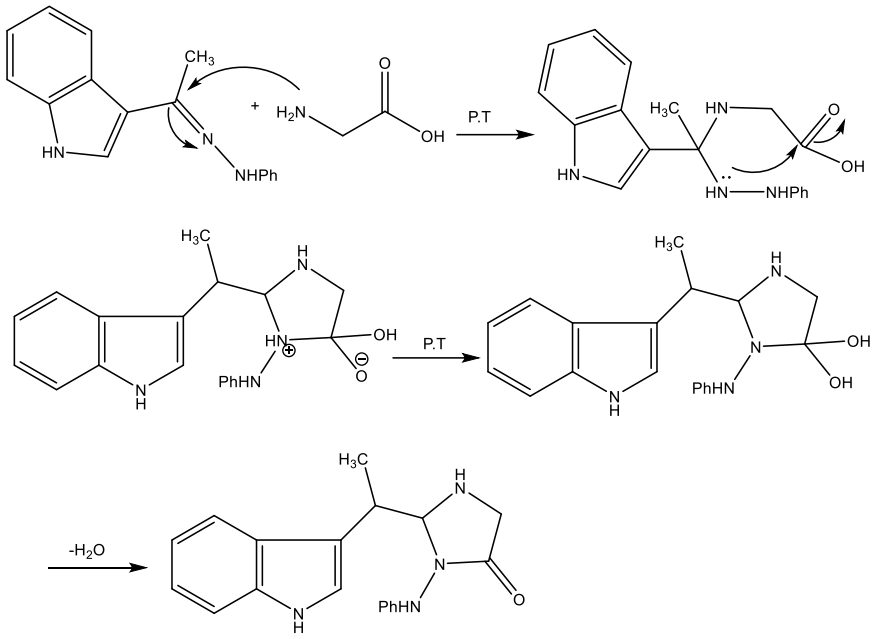

Figure 4. Formation of compound **4**

2-(*1H*-indol-3-yl)-2-methyl-3-(phenylamino) thiazolidin-4-one **3** was underwent condensation with benzaldehyde to obtain 5-benzylidene-2-(*1H*-indol-3-yl)-2-methyl-3-(phenylamino) thiazolidin-4-one **5** scheme **1**. The reactions were performed using ethanol under reflux in the presence of piperdine. ^{1}H-NMR spectrum characterized compound **5** by appearance a signal as a singlet for methine proton (-CH-) in the range of 7.87 ppm and disappearance of signals the corresponding singlet of two methylene (–CH_2–) protons was shown by compound **5**, verified by ^{13}C-NMR of compound **5** was appeared a peak at 166.5 ppm established to carbon atom (C=O) of thiazolidinone ring and another carbon at 76.9, 125.8 and 138.6 ppm of thiazole ring.

b) Condensation of 3-acetylindole with hydrazine hydrate

Reaction of 3-acetyl indole **1** with hydrazine hydrate by equivalent weight (1:1 Molar ratio, yielded the corresponding hydrazone **6** [8] Scheme 2. The IR spectrum of compound **6** showed disappearance of ($C=O_{acetyl}$) absorption band which was observed at 1608 cm^{-1} also, new absorption band attributable to NH_2 group appearance at 3342 and 3154 cm^{-1} and another absorption band at 1631cm^{-1} due to (C=N). The free amino group in compound **6** was subjected to formation Schiff base reactions with some carbonyl compounds. Therefore, the reaction of hydrazone **6** with 2-thiophene aldehyde in absolute ethanol under reflux for 2

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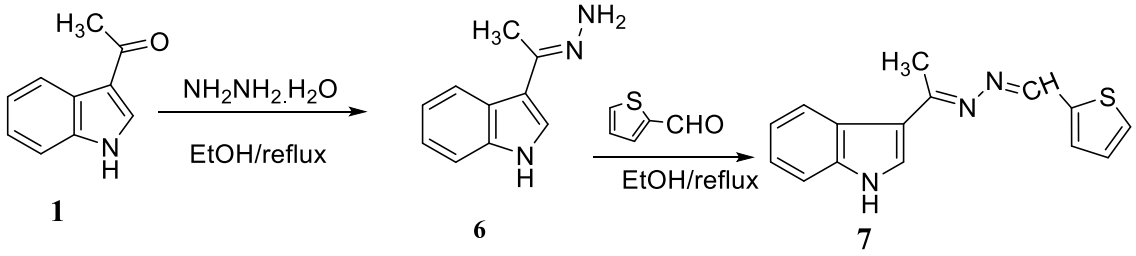
hours, afforded hydrazone **7** Scheme 2**.** ^{1}H-NMR spectrum was showed a singlet signal assignable to CH=N appeared at chemical shift 8.78 ppm and not found a singlet signal at 6.96 ppm which represents NH_2 group. ^{13}C-NMR of compound **7** was appeared a peak at 139.6 ppm established to carbon atom (CH=N) and another carbon at 162.1 ppm of (C=N) group.



Scheme 2.

In the same method, hydrazone **6** was condensed with isatine and/or 3-acetylindole **1** afforded the hydrazone **8** and **9** respectively Scheme 3. IR spectrum of compound **8** showed absorption bands at 1675 cm^{-1} assignable to carbonyl group and at 1608 for (C=N). In the mass spectrum of compound **8**, the molecular ion peak was recorded at m/z 344 which agrees well the expected molecular formula weight (344.36). IR spectrum of compound **9** showed absorption bands also, at 1610 cm^{-1} represented (C=N) and 3196 (NH).

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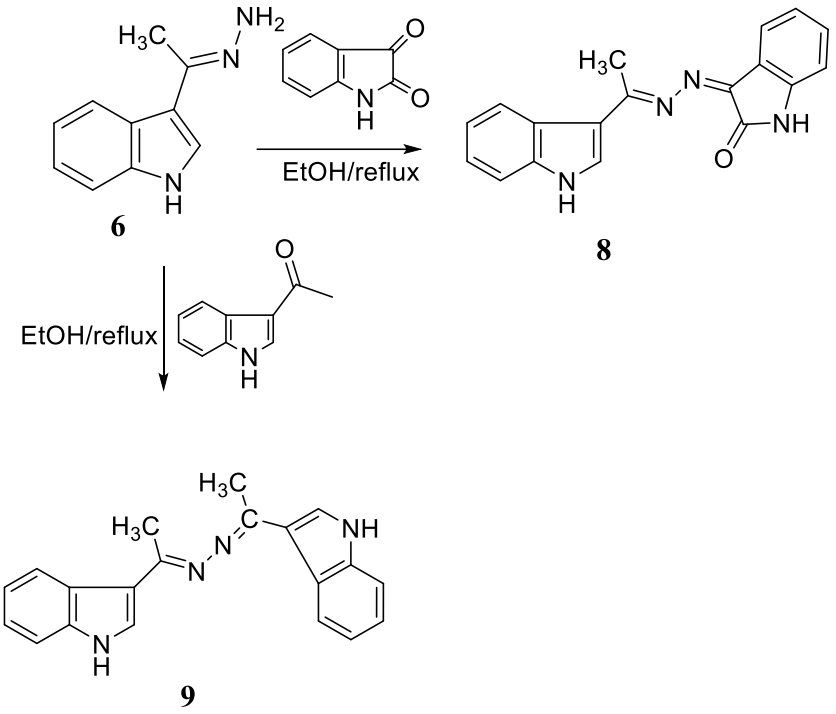

Scheme **3.**

B. Reaction of 3-acetyl indole with thiosemicarbazide.

1-(*1H*-Indol-3-yl ethylidene) thiosemicarbazide **10** [9] has been synthesized through condensation between 3-acetylindole **1** and thiosemicarbazide in the presence of *p*-toluenesulfonic acid (*p*TsOH) used as a catalyst Scheme 4. The structure of the synthesized compound **10** was confirmed by thin layer chromatography and was characterized by UV spectrum at 204 nm (CH=CH) corresponding to the Ţ-t transition of aromatic double bond and a peak at 330 nm is due to n-t* transition which clearly indicates the presence of indoyl moiety . IR spectrum of compound **10** was showed a weak absorption band at 3120 cm^{-1} was assigned the presence of C-H stretching vibration in aromatic system and other absorption band at 3419 cm^{-1} due to the NH_2 stretching but at 1610 $cm^{-1}$was founded a sharp peak due to C=N bond and a absorption band at 1242 cm^{-1} was detected the presence of C=S stretching frequency.

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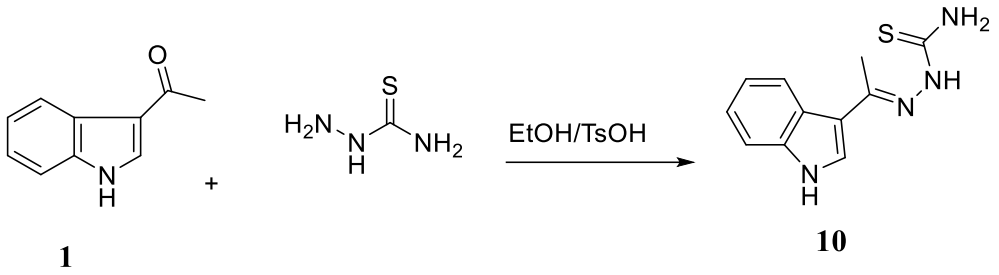

1 **10**

Scheme **4.**

In this section, we used thiosemicarbazone as a precursor for the synthesis of a variety of bioactive sulfur-nitrogen heterocyclic systems as known [10, 11]. Therefore, Compound **10** was treatment with concentrated hydrochloric acid to cyclization carbothioamide into 5-(*1H*-indol-3-yl)-5-methyl-4,5-dihydro-1,3,4-thiadiazol-2-amine **11**. IR spectrum of compound **11** was characterized by the presence of strong bands at 3373, 3156 cm^{-1} due to NH_2, NH. ^{1}H-NMR spectrum was showed a singlet signal at δ 8.17 ppm due to (NH_2) group and showed the NH proton of thiadiazole at δ 8.27 ppm. Furthermore, compound **10** was refluxed with acetic anhydride for 7 hr., gave the corresponding compound **12** Scheme 5. Compound **12** may be formed via acetylation of NH_2 moiety and azomethin. The compound **12** was determined on the basis of spectral data. IR spectrum of compound **12** was elucidate the presence of C=O of acetyl group at ν1707 and 1653 cm^{-1}. ^{1}H-NMR spectrum of compound **12** confirmed two singlet signals at δ 1.64 and 2.42 ppm assigned to protons of methyl groups, in addition a singlet at δ 11.88 ppm corresponding to ($NHCOCH_3$) and ^{13}C-NMR spectrum was also a good support for the determined the structure of **12** which appearance the signals at δ 149.8 for (C=N) and at 24.4, 28.8 ppm corresponding to carbon atoms of two methyl group, in addition to carbonyl group appearance at δ169.0 for ($NCOCH_3$) and δ 174.8 due to ($NHCOCH_3$) ppm.

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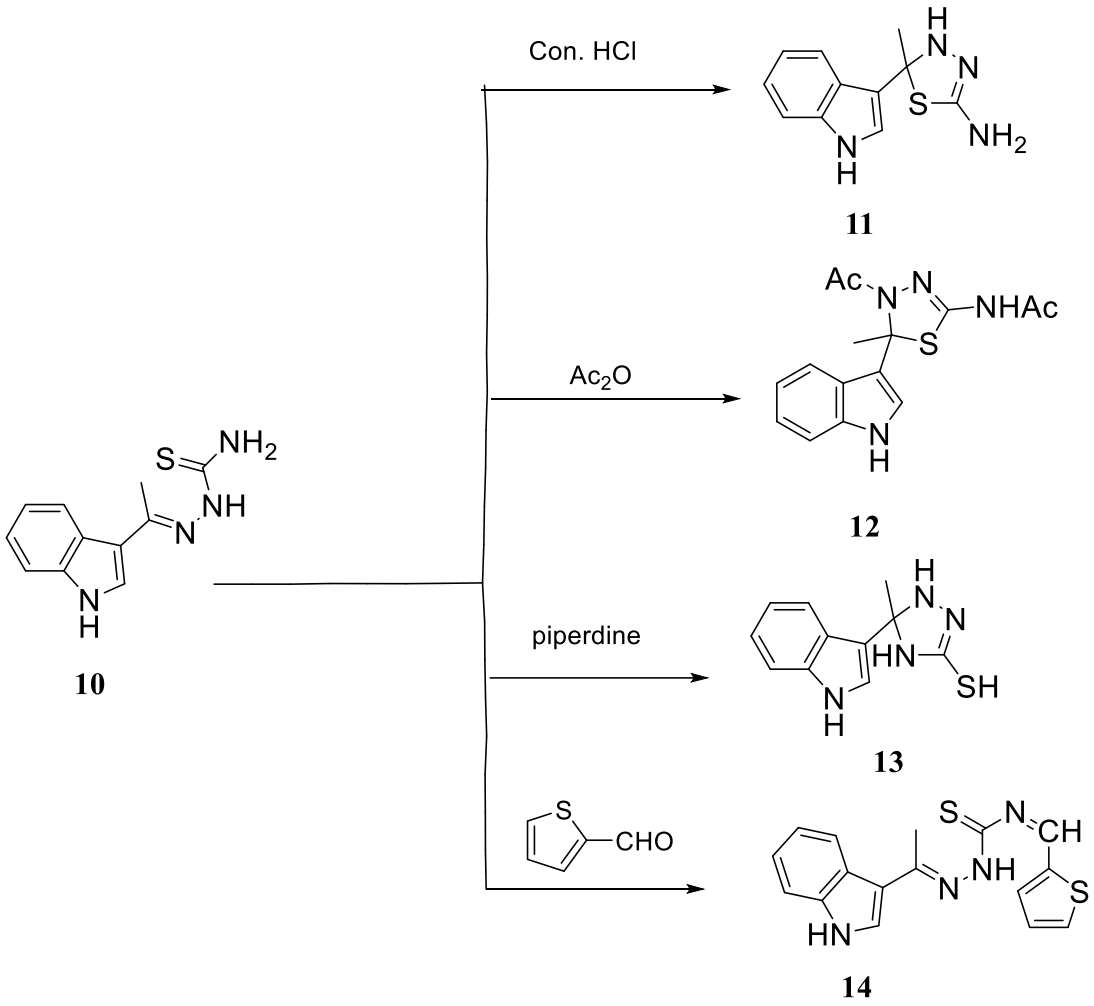

Scheme 5.

On the other hand, the cyclization of compound **10** in the presence of piperdine produced compound **13** Scheme 5, which was confirmed on the basis spectral data. IR spectrum for compound **13** showed stretching vibration band at 1612 cm^{-1} characteristic for the C=N and at 3125 cm^{-1} for NH. ^{1}H-NMR spectrum of compound **13** appearance a signal as a broad singlet at δ13.86 ppm assigned to proton of –S-H.

Thiosemicarbazide derivative **10** has been condensed with thiophene-2-carbaldehyde in ethanol in presence of few drops of glacial acetic acid to give 2-(1-(*1H*-indol-3-yl) ethylidene)-*N*-thiophen-2-ylmethylene) hydrazine-1-carbothioamide **14** Scheme 5.The IR spectrum of compound **14** revealed the presence of absorption bands at 1610 (C=N), 1242 (C=S) cm^{-1}. ^{1}H-NMR spectrum of the product **14** revealed the presence of (N=CH-) as a characteristic singlet at δ 8.82 ppm, in addition to the signal assigned to (NH-C=S) at δ10.07

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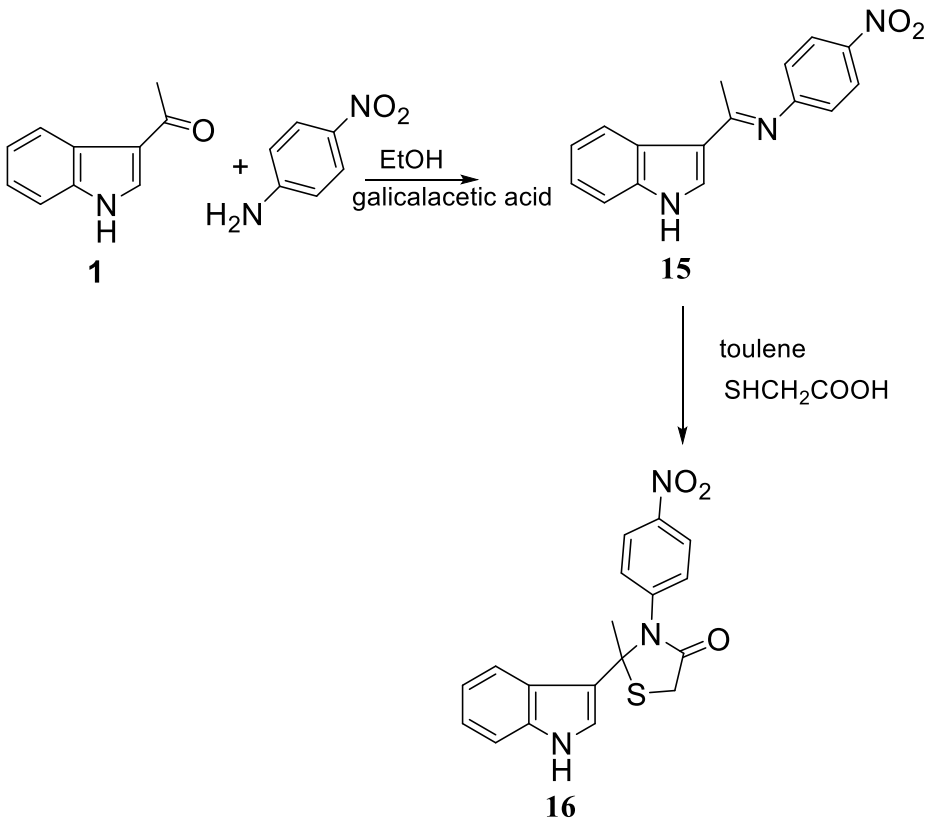
ppm. Its ^{13}C-NMR spectrum showed the signal at 193.1 for C=S) in addition to the presence of signal at 156.3 for (N=CH).

2-Reaction of 3-acetyl indole with amines compounds

3-Acetylindole **1** was treated with *p*-nitroaniline to produce corresponding 1-(*1H*-indol-3-yl)-*N*-(4-nitrophenyl)ethanimine Schiff bases **15**. Then, compound **15** was reacted with thioglycolic acid refluxing in dry toluene for 6 hr to afford thiazolidinone derivatives **16** Scheme 6**.** IR spectrum of compound **15** revealed absorption band at 1627 due to (C=N) group and the nitro group appearance at 1315-1442cm^{-1}. The IR spectrum of compound **16** showed characteristic absorption band at 1635 cm^{-1} assigned to carbonyl group of thiazolidinone ring and another absorption band at 133-1512cm^{-1} due to the (NO_2) group.



Scheme 6.

Moreover, treating 3-acetylindole **1** with ethylenediamine in 2:1 molar ratio refluxing in absolute ethanol afforded the bis imine indole **17** Scheme 7**.**

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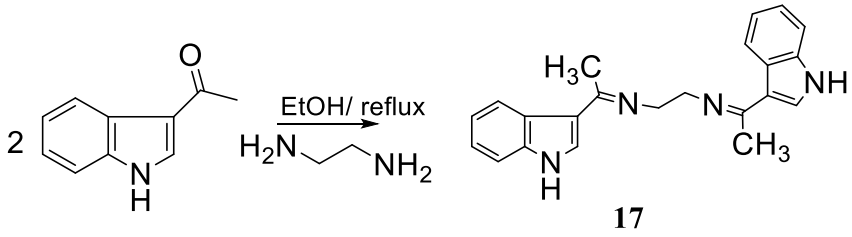
The molecular ion peak was recorded at m/z 342 which agrees well the expected molecular formula weight (342.45).



17

Scheme 7.

^{13}C-NMR of compound **17** appearance signal of two methyl group at δ24.3 ppm and at 163.6 due to (2C=N) and another signal at 63.1 indicated ($2CH_2$), in addition to the absence of the signal in downfield region at 194.3 for carbonyl of 3-acetylindole.

Results of antibacterial activity.

The antibacterial assay of synthesized compounds was screened against two gram positive bacteria (*S.aureus* and *Bacillus cereus*) and three gram negative bacteria (*E.coli*, *K.pneumoniae*, and *P.aeruginosa*) using agar well diffusion method. Table 1 shows that most of the tested compounds had an inhibitory effect on growth of gram-positive bacteria with **8**, **11** and **14** as the most active ones. For *S.aureus* bacteria, compounds' activity was arranged in the following order **8** > **5**, **14** > **3**> **13** and no inhibition zones were recorded for **11**. For *B.cereus*, compounds' activity was arranged as follows **8** > **5** > **3** > **14** > **11**, **13** figure 1.

Screening the antibacterial potential against Gram-negative isolates indicated that **5** showed moderate activity and nearly same inhibition zone of the standard antibiotic against *E.coli* while **13** was inactive. For *K.pneumoniae*, four compounds had weak inhibitory activity (**8**, **11** and **14**) while the rest of compounds were completely inactive. None of the tested compounds had activity against *P.aeruginosa*, see figure 2.

Table (1) antibacterial activities of tested compounds[a,b]

Compd No	**Bacterial isolates**									
	S.aureus		***B.cereus***		***E.coli***		***K pneumoniae***		***P.aeruginosa***	
	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI
3	15	0.48	13	0.43	14	0.78	-	0	-	0

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5	19	0.61	20			1	-	0	-	
8	20	0.65	21	0.7	13	0.72	11	0.39	-	0
11	-	0	12	0.4	11	0.6	15	0.54	-	0
13	13	0.42	12	0.4	-	0	-	0	-	0
14	19	0.61	14	0.47	13	0.72	11	0.39	-	0
Gentamicin	31	1	30	1	18	1	28	1	26	1

S.aureus: Staphylococcus aureus; B. cereus: Bacillus cereus; E. coli: Escherichia coli; Pseudomonas aeruginosa: P. aeruginosa; Klebsiella pneumoniae:K.pneumoniae.

IZ: inhibition zone, AI: activity index

[a] Sample concentration: 5mg/ml, sample volume 100 µl/ml

[b] Inhibition zone diameters were calculated after subtraction of solvent activity

Results are calculated after subtraction of solvent activity (DMSO and DMF)

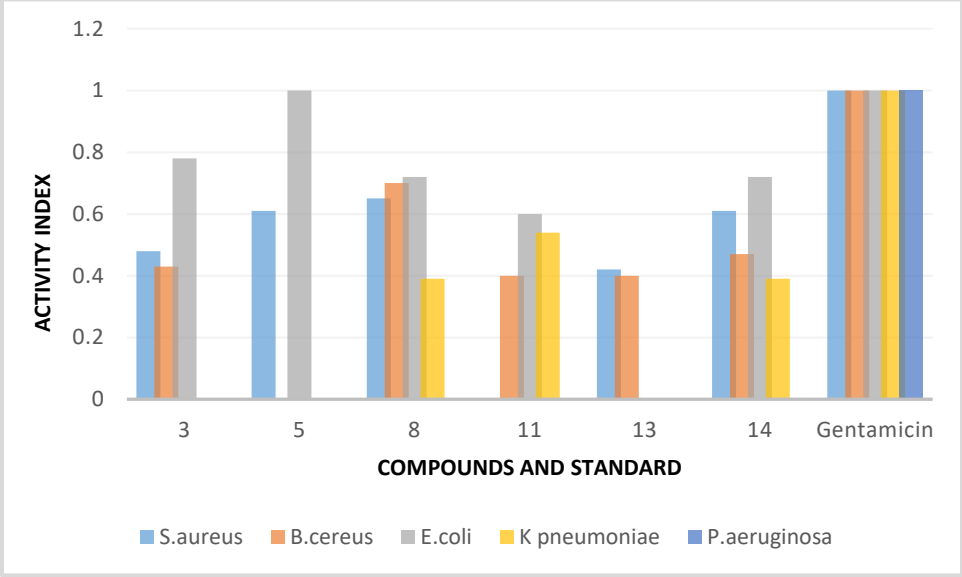

Figure 5.

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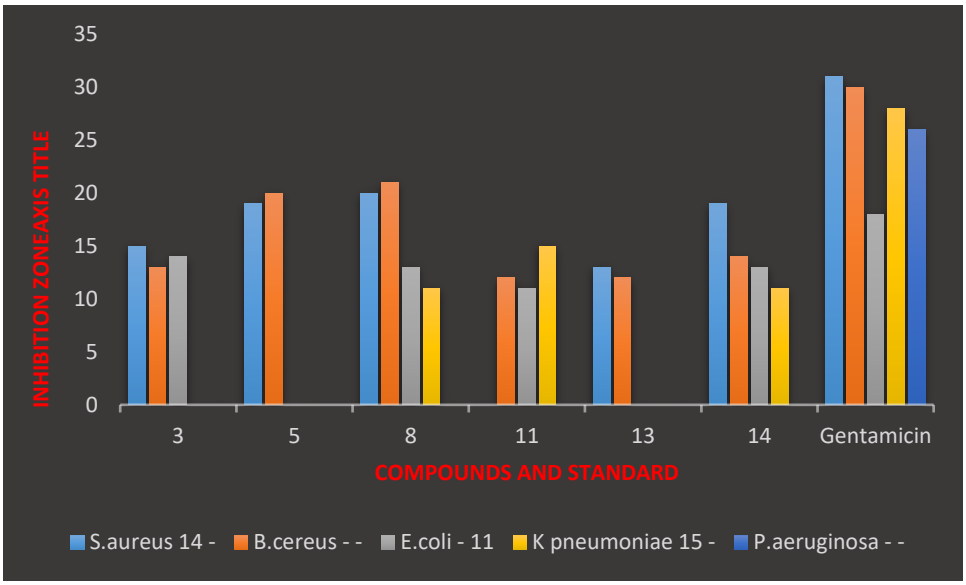

Figure 6.

Antioxidant & anti-inflammatory assay in *vivo*

Serum MDA level as an oxidative stress parameter in the studied groups:

Table (1) showed that there was significant increase in serum Malondialdehyde (MDA) level in inflammatory group (group II) compared to the control (group I) and other treated inflammatory groups (group III subgroups A, B, C & D) except group III subgroup E that has no significance difference as compared with (group II) that clarifies that **7** compounds have no significant role in eliminating the oxidative stress. On the other hand, there were no significant differences between (group III subgroups A & B) and the control (group I) which emphasizes the significant therapeutic effects of **4** & **14** compounds to eliminate the oxidative stress. Additionally, there were significant differences between (group III subgroups C & D) and the control (group I) verifying the moderate effects of **8** & **13** compounds in abolishing the oxidative stress.

Serum catalase activity as an antioxidant enzyme in the studied groups:

Table (2) showed that there was significant decrease in serum catalase activity in inflammatory group (group II) compared to the control (group I) and other treated inflammatory groups (group III subgroups A, B, C & D) except group III subgroup E that has no significance difference as compared with (group II) that clarifies that **7** compounds have no significant role in activation of antioxidant enzymes. However, there were no significant differences between (group III subgroups A & B) and the control (group I) which emphasizes the significant therapeutic effects of **4** & **14** compounds to activate the antioxidant enzymes. In addition, there were significant differences

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between (group III subgroups C & D) and the control (group I) verifying the moderate effects of **8** & **13** compounds in activation of the antioxidant enzymes

Total antioxidant capacity (TAC) in the studied groups:

Table (3) showed that there was significant decrease in serum total antioxidant capacity in inflammatory group (group II) compared to the control (group I) and other treated inflammatory groups (group III subgroups A, B, C & D) except group III subgroup E that has no significance difference as compared with (group II) that clarifies that **7** compounds have no significant role in activation of total antioxidant capacity. Conversely, there were no significant differences between (group III subgroup A) and the control (group I) which emphasizes the significant therapeutic effect of **4** compounds in activation of total antioxidant capacity. Furthermore, there were significant differences between (group III subgroups B, C & D) and the control (group I) verifying the moderate effects of **14**, **8** & **13** compounds in activation of total antioxidant capacity.

Renal toxicity in the studied groups:

Table (4) showed that there were no significant differences in serum creatinine levels between inflammatory group (group II), the control (group I) and other treated inflammatory groups (group III subgroups A, B, C, D & E). Serum creatinine levels were in the normal ranges. In addition, there was significant difference in blood urea nitrogen (BUN) level between inflammatory group (group II) and the control (group I). Also, there was significant difference between inflammatory group (group II) and (group III subgroup A). Yet, all BUN levels in all studied groups were in the normal ranges that clarifies that no renal toxicity for the induction compound (carrageenan) ant all prepared compounds used as treatment.

Liver toxicity in the studied groups:

Table (5) showed that there was significant increase in liver enzymes activities in (group II, group III subgroups C, D & E) compared to the control (group I) ensuring the hepatic toxicity of the induction compound (carrageenan) and **8**, **13** & **7** compounds used as treatment. Moreover, group III subgroup E showed significant increase in liver enzymes activities as compared with in inflammatory group (group II) indicating the exaggerated hepatic toxicity by using **7** compounds as treatment. In contrast, there were no significant differences between (group III subgroups A &

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B) and the control (group I) which emphasizes the significant therapeutic effects of **4** & **14** compounds in treatment of hepatic toxicity induced by carrageenan.

Serum NF-κ$^{\beta}$ Level as an inflammatory mediator in the studied groups:

Table (6) showed that there was significant increase in serum **NF-κ$^{\beta}$** Level in inflammatory group (group II) compared to the control (group I) and other treated inflammatory groups (group III subgroups A, B, C) except group III subgroup D & E that have no significance differences as compared with (group II) that clarifies that **13** & **7** compounds have no significant role in eliminating of the inflammatory mediators. On the other hand, there were no significant differences between (group III subgroup A) and the control (group I) which emphasizes the significant therapeutic effects of **4** compounds to eliminate the inflammatory mediators. Additionally, there were significant differences between (group III subgroups B, C & D) and the control (group I) verifying the moderate effects of **14**, **8** & **13** compounds in eliminating of the inflammatory mediators.

Table (1): Oxidative stress parameter in the studied groups:

Parameter/ Groups	**Group I (n=10)**	**Group II (n=10)**	**Group III**					**F value**	**P-value**
			Subgroup A (n=10)	**Subgroup B (n=10)**	**Subgroup C (n=10)**	**Subgroup D (n=10)**	**Subgroup E (n=10)**		
Serum MDA (nmol/ml)	**2.06 ±0.3**	**7.02 ±0.5** [a]	**2.58± 0.6** [b]	**2.84 ±0.5** [b]	**3.31 ±0.9** [a,b]	**5.6 ±1.1** [a,b,c,d,e]	**6.23 ±0.9** [a,c,d,e]	**73.9**	**< 0.001***

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Parameter/Groups	**Group I (n=10)**	**Group II (n=10)**	**Group III**					**F value**	**P-value**
			Subgroup A (n=10)	**Subgroup B (n=10)**	**Subgroup C (n=10)**	**Subgroup D (n=10)**	**Subgroup E (n=10)**		
Serum Catalase (U/L)	**69±2.9**	**28.6±4.9** [a]	**61.1±7.9** [b]	**50.9±3.9** [a,b,c]	**44.5±8.9** [a,b,c]	**38.1±7.1** [a,b,c,d]	**30.3±4.3** [a,c,d,e]	**62.12**	**<0.001***

Values are expressed as mean ± SD. Number of rats in each group (n=10). P was considered significant at <0.05. a Significance vs. **group I**, b Significance vs. **group II**, c Significance vs. **Group III** (**subgroup A)** d Significance vs. **Group III** (**subgroup B)**, e Significance vs. **Group III** (**subgroup C)**, using One way ANOVA followed by Tukey's post hoc test for multiple comparison.

Table (2): Antioxidant enzyme activity in the studied groups:

Values are expressed as mean ± SD. Number of rats in each group (n=10). P was considered significant at <0.05. a Significance vs. **group I**, b Significance vs. **group II**, c Significance vs. **Group III** (**subgroup A)** d Significance vs. **Group III** (**subgroup B)**, e Significance vs. **Group III** (**subgroup C)**, using One way ANOVA followed by Tukey's post hoc test for multiple comparison.

Table (3): Total antioxidant capacity in the studied groups:

Parameter/Groups	Group I (n=10)	Group II (n=10)	Group III					F value	P-value
			Subgroup A (n=10)	Subgroup B (n=10)	Subgroup C (n=10)	Subgroup D (n=10)	Subgroup E (n=10)		
Serum TAC (mmol/ml)	0.8±0.07	0.37±0.06 [a]	0.7±0.09 [b]	0.62±0.04 [a,b,c]	0.57±0.04 [a,b,c]	0.49±0.06 [a,b,c,d]	0.43±0.08 [a,c,d,e]	67.7	< 0.001*

Values are expressed as mean ± SD. Number of rats in each group (n=10). P was considered significant at <0.05. a Significance vs. **group I**, b Significance vs. **group II**, c Significance vs. **Group III** (**subgroup A)** d Significance vs. **Group III** (**subgroup B)**, e Significance vs. **Group III** (**subgroup C)**, using One way ANOVA followed by Tukey's post hoc test for multiple comparison.

Table (4): Renal toxicity in the studied groups:

Parameter/Groups	**Group I (n=10)**	**Group II (n=10)**	**Group III**	**F value**	**P-value**

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			Subgroup A (n=10)	Subgroup B (n=10)	Subgroup C (n=10)	Subgroup D (n=10)	Subgroup E (n=10)		
Serum creatinine (mg/dl)	**0.62±0.14**	**0.78±0.3**	**0.72±0.09**	**0.74±0.1**	**0.84±0.23**	**0.69±0.25**	**0.69±0.28**	**1.07**	**0.39**
BUN (mg/dl)	**9.7±1.6**	**12.5±4.7** [a]	**11.3±2.2** [b]	**11.9±2.2**	**12.1±2.4**	**12.8±2.9**	**12.6±3.2**	**3.6**	**0.004***

Values are expressed as mean ± SD. Number of rats in each group (n=10). P was considered significant at <0.05. a Significance vs. **group I**, b Significance vs. **group II**, using One way ANOVA followed by Tukey's post hoc test for multiple comparison.

Table (5): Liver toxicity in the studied groups:

Parameter/Groups	Group I (n=10)	Group II (n=10)	Group III					F value	P-value
			Subgroup A (n=10)	Subgroup B (n=10)	Subgroup C (n=10)	Subgroup D (n=10)	Subgroup E (n=10)		
Serum ALT (U/L)	**23.7±3.27**	**70.8±12.09** [a]	**27.6±5.7** [b]	**33.2±6.1** [b]	**75.2±13.6** [a,c,d]	**75.1±12.8** [a,c,d]	**86.2±10.5** [a,b,c,d]	**69.6**	**< 0.001***
Serum AST (U/L)	**20.7±3.7**	**69.8±13.1** [a]	**26.6±5.3** [b]	**31.2±4.8** [b]	**74.2±13** [a,c,d]	**74.1±13.1** [a,c,d]	**84.2±11.8** [a,b,c,d]	**67.87**	**< 0.001***

Values are expressed as mean ± SD. Number of rats in each group (n=10). P was considered significant at <0.05. a Significance vs. **group I**, b Significance vs. **group II**, c Significance vs. **Group III** (**subgroup A)** d Significance vs. **Group III** (**subgroup B)**, using One way ANOVA followed by Tukey's post hoc test for multiple comparison.

Table (6): Serum NF-κ$^{\beta}$ Level (inflammatory mediator) in the studied groups:

Parameter/Groups	Group I (n=10)	Group II (n=10)	Group III					F value	P-value
			Subgroup A (n=10)	Subgroup B (n=10)	Subgroup C (n=10)	Subgroup D (n=10)	Subgroup E (n=10)		
Serum NF-κβ (ng/ml)	**0.4±0.8**	**2.03±0.1** [a]	**0.47±0.13** [b]	**0.74±0.3** [a,b,c]	**1.2±0.2** [a,b,c,d]	**1.77±0.3** [a,c,d,e]	**2 ±0.14** [a,c,d,e]	**134.5**	**< 0.001***

Values are expressed as mean ± SD. Number of rats in each group (n=10). P was considered significant at <0.05. a Significance vs. **group I**, b Significance vs. **group II**, c Significance vs. **Group III** (**subgroup A)** d Significance vs. **Group III** (**subgroup B)**, e Significance vs. **Group III** (**subgroup C)**, using One way ANOVA followed by Tukey's post hoc test for multiple comparison.

Molecular docking

For better identification of the approach in which ligand interacts with receptor and perform particular function, docking studies were carried out. The results were elaborated by

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employing the Pdb IDs of crystal structures of *Escherichia Coli* glutathione transferase protein as shown in **Figure 7** as macromolecules.

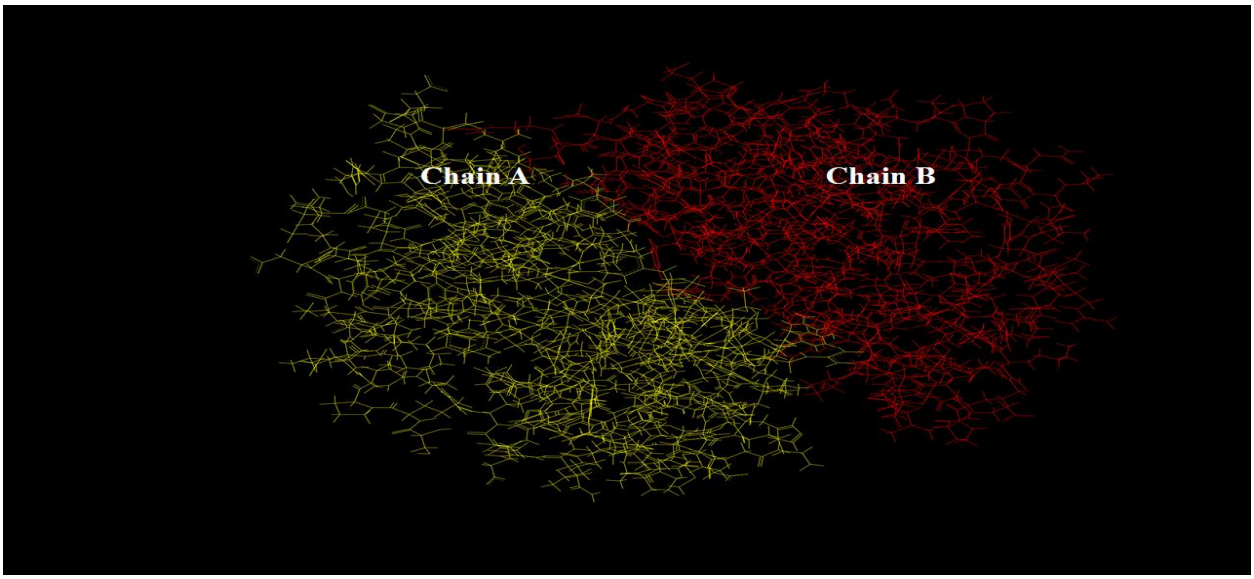

Figure 7: Crystallographic image of *Escherichia Coli* glutathione transferase protein having Chain A and Chain B with Pdb ID: 5HFK

All the docking results were compiled to recognize the different parameters of interaction. Computational studies adopted a definite position in calculating and scrutinizing the right way of binding of ligands into active site. Docking studies of compound **8** showed excellent result against *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeroginosa* with negative binding energies -9.5, -8.2, -8.5, -7.2 and -8.6 Kcal/mol respectively in best interacting posture. Docking of compound **11** with *E coli* glutathione transferase protein showed promising results as it interacts with both the chains (A and B) of protein as shown in **Figure 8.** It gave the lowest binding energy of -7.1 Kcal/mol in best binding posture with persistent values of -6.9 to -6.7 till posture 9 that indicates the good affinity of this ligand with receptor. Looking into the figure 2, all possibilities of interaction can be analyzed. The heteroatom nitrogen plays a fundamental role by forming two conventional hydrogen bonds with GLY A: 106 and VAL B: 105 at the bond distance of 2.60 Å (angstrom) and 2.64 Å respectively and appears to be a linker between two chains of protein. Furthermore aromatic phenyl ring performed by forming π-alkyl linkage with MET A: 111. Other important residues were found to be GLU B: 71, GLY B: 73, LYS B: 14, PHE

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A: 102, TRP B: 169, GLY B: 109, GLN B: 114, GLY A: 107, ARG A: 139 and Pro B: 110. Some unfavorable donor-donor linkage also appeared with SER B: 72 and ASN B: 49.

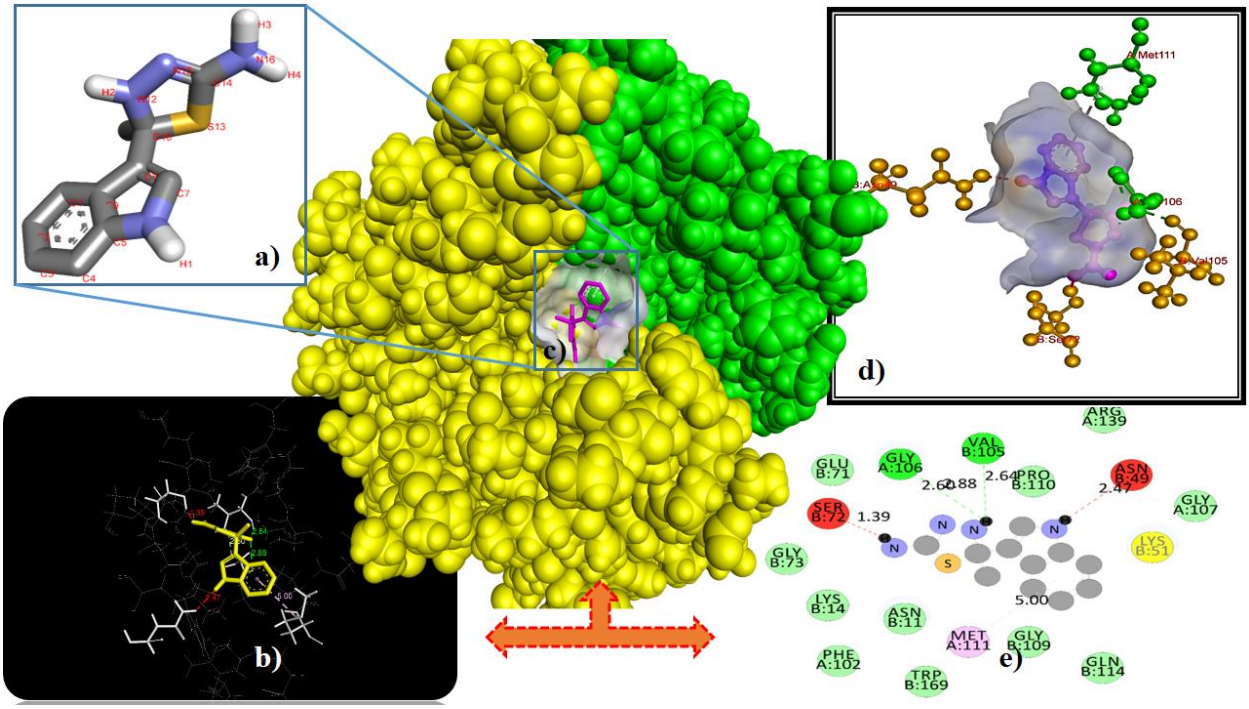

Figure 8: Synthesized compound **11** having inhibitory potential against *Escherichia Coli*, **a)** 3d visualization of compound **11** elements shown, **b)** binding pocket amino acids (white) participating with interaction with synthesized compound **11** (yellow)**, c)** 3d display of protein chain A and B along with inner view of compound **11, d)** Green colour amino acids of chain A and brown color amino acids of chain B, **e)** 2d image with bonding interactions measurements

The interaction analysis of compound **8** with all bacterial strains especially *Bacillus cereus* indicated that this ligand has the capacity to be a prominent candidate as anti-bacterial agent. It showed the binding affinity with negative energy of -8.5 Kcal/mol along with 37.359 rmsd /ub and 36.258 rmsd/lb. Results have been enumerated in **Figure 9**. Prominent interactions were pi-pi T-shaped and Pi anion interaction that appears between phenyl and two protein building blocks GLU A: 57 and TRP A: 59 with bond distance of 3.65 Å (angstrom) and 4.88 Å respectively. Other amino acids were found to be ALA A: 175, HIS A: 210 and VAL A. Carbon-hydrogen bond was not prominent in this interaction. In addition to all these, pi-pi

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stacked and pi-alkyl interactions seemed with ALA A: 175 and VAL A: 39 accordingly. Nitrogen atom also formed a fundamental role in linkages.

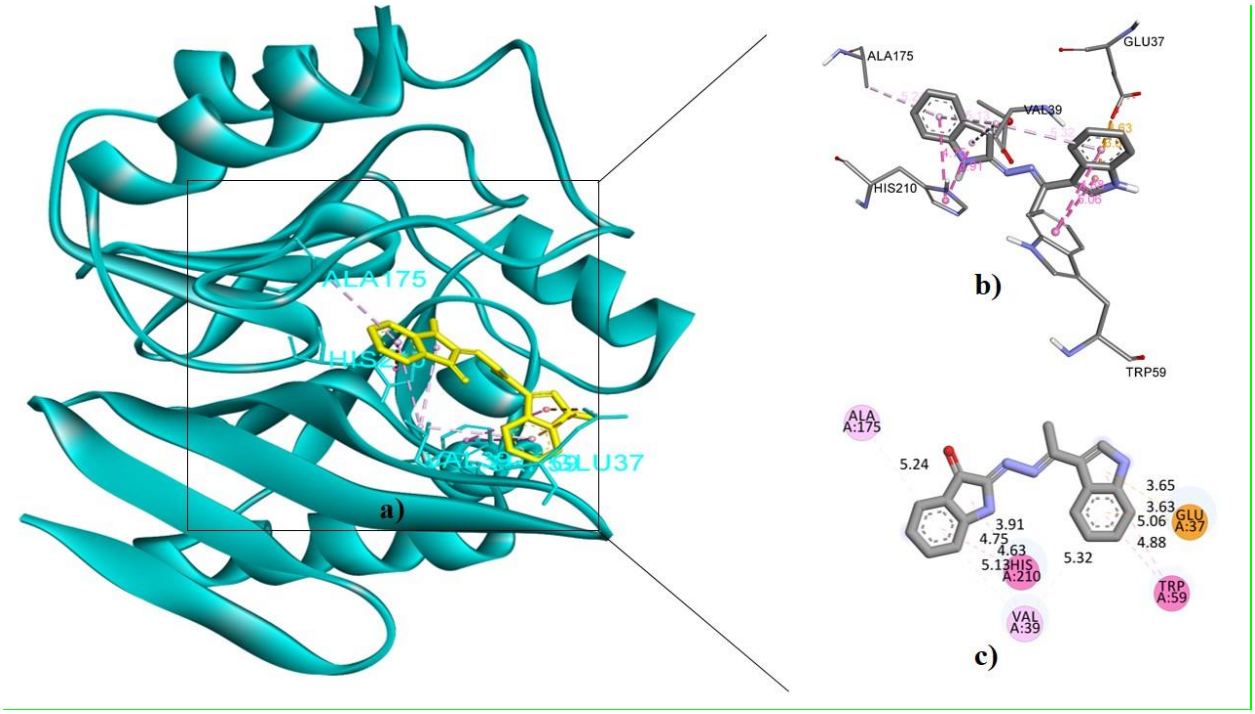

Figure 9: a) DSV 3d visualization of compound **1** (brown) with protein (blue) of *Bacillus Cereus* Bacterial specie, **b)** Interactions with specific amino acids labelled, **c)** Two dimensional view.

Interaction analyses of compound **5** gave important findings about clear picture of its attachment with glutathione transferase protein of *Escherichia Coli*. Impression of results are shown in **Figure 10**

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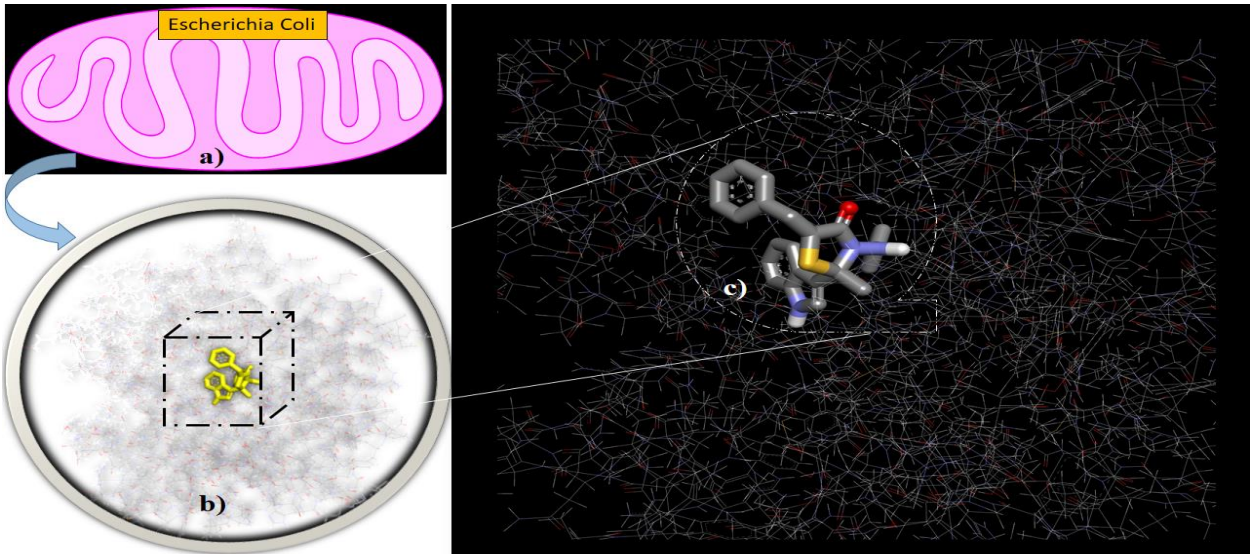

Figure 10: a) *Escherichia Coli* dummy view, **b)** Microscopic vision of protein with compound **7** inside, **c)** Zooming out from inner sight.

Remarkable results were achieved with docking values of -9.2 Kcal/mol that ended up at -7.7 Kcal/mol binding energies in the best 9 postures. Interactions were additionally interpreted for analyzation of bond types and distances. Docking results as given in **Figure 11** indicated that three conventional hydrogen bonds were involved in interacting the ligand to protein. These bonds appeared with ASN A: 11, LYS A: 51 and GLY A: 109 having bond lengths of 2.60 Å, 2.53 Å and 2.47 Å respectively. To make this bond stronger, two pi-cation linkage appeared between two ligand rings phenyl, heterocyclic and one amino acid LYS A: 51 having bond measurement of 4.80 Å and 4.40 Å. Pro A: 110 was another important building residue that formed pi-alkyl linkage with bond length of 4.26 Å.

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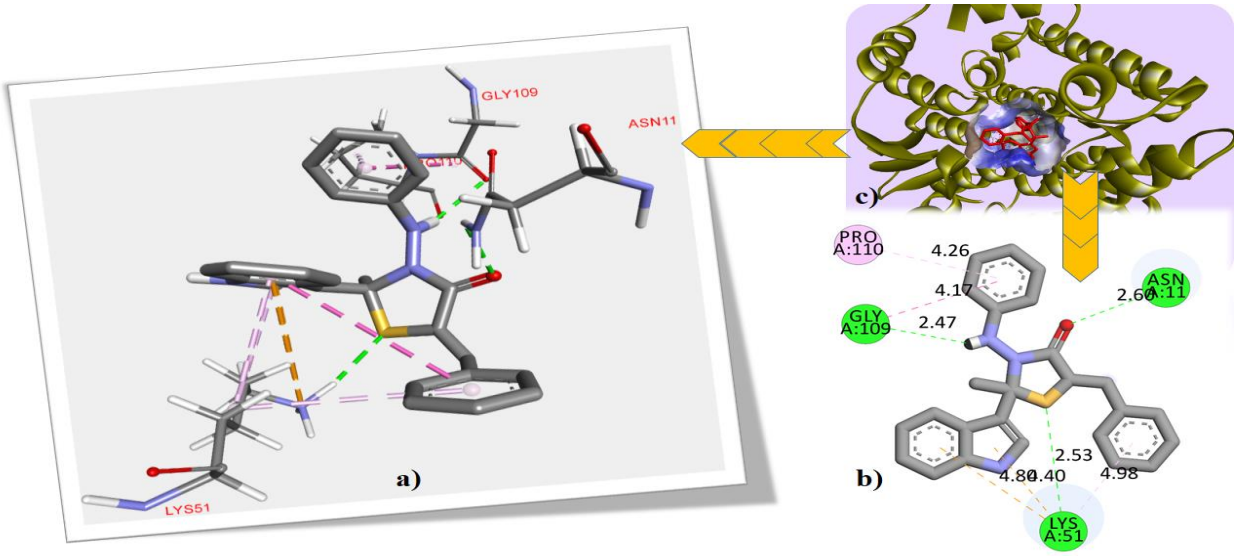

Figure 11: a) Discovery studio view of compound **5** within the *Escherichia Coli* glutathione transferase protein, **b)** Two dimensional view, **c)** Active site exposed with compound **5** inside**.**

Similarly, interactions studies of compound **8** with *Staphylococcus aureus* protein with Pdb Id: 1VQQ have impressive findings as elaborated in **Figure 12**. Results indicated that this ligand has excellent capability of inhibiting the bacterial strains as it have the negative binding affinity energy values in the range of -9.5 Kcal/mol to -8.0 kcal/mol. As explained in two dimensional image that this compound has three phenyl rings with two heterocyclic ring that modified the way of its attachment to receptors. Two main type of bonds were pi-anion and pi-alkyl. A huge number of van der Waals interactions appeared with LYS B: 68, LYS A: 68, ILE A: 69, SER B: 72, SER A: 306, ASN A: 307, ILE B: 142, SER A: 141, ILE A: 142 and ASN B: 307. ASP B: 304, ARG A: 65, ASP A: 304 and ARG B: 65 were attached through pi-anion linkages showing bond length of 3.56Å, 401Å, 3.95Å and 4.29Å respectively. ILE B: 69 was found attached through pi-alkyl and SER B: 141 through Carbon hydrogen bond individually.

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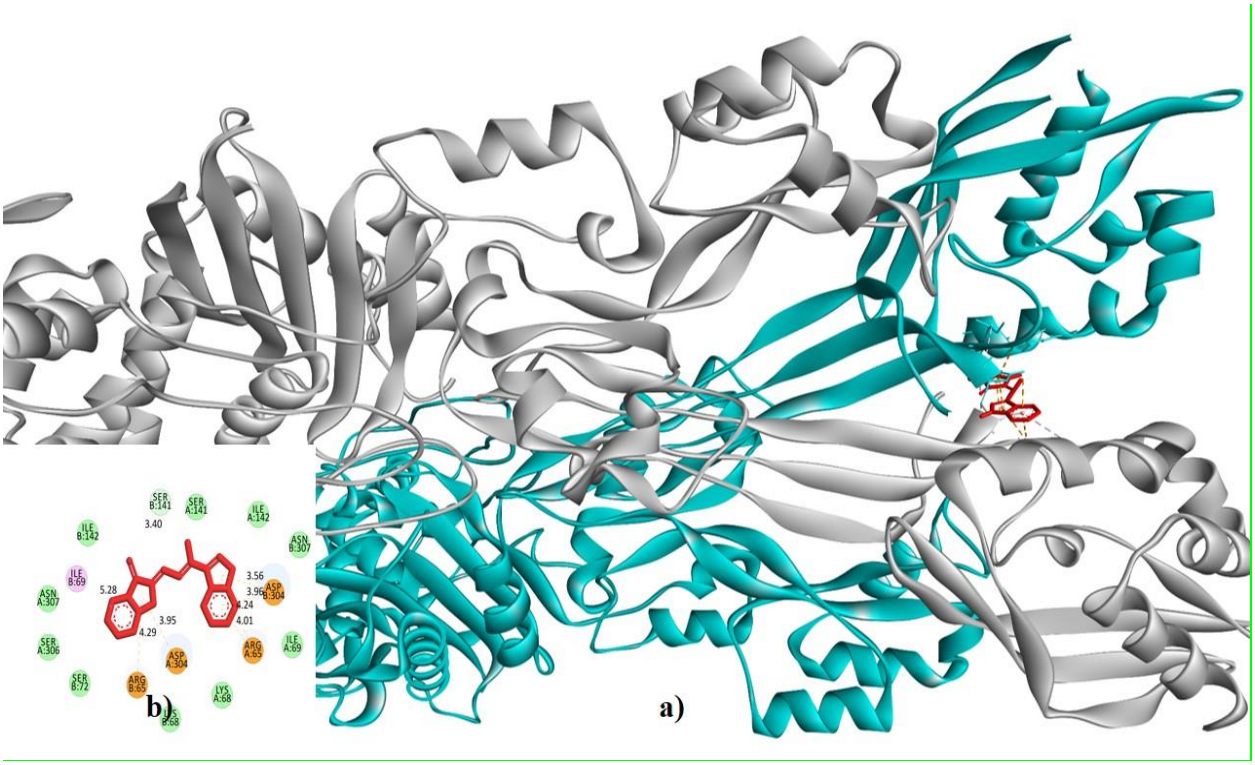

Figure 12: A. *Staphylococcus aureus* (Chain A in grey & Chain B in blue) with compound 1 (red) three dimensional image, **B**. Two dimensional picture inside binding pocket

Conclusion

In this study, synthesis of some novel 3-substitued indole derivatives using using 3-Acetyl indole **1** as a starting material. All new synthesized compounds were screened against their *in vitro* antibacterial activity and screened their *in vivo* antimicrobial and antioxidant activity. Structures of the newly synthesized compounds examination by spectral data (IR, ^{1}H- NMR and ^{13}C -NMR).

Experimental

Thomas Hoover Electronic Apparatus of melting point measurements were hand-me-down for determination of the (melting points) stated by the following work. Determinations of infrared bands were done and documented as a KBr picture using FTIR Shimadzu (Japan), in University of Al-Jouf, at college of science. ^{1}H-NMR spectra and ^{13}C -NMR (solvent DMSO-*d6*) were recorded on 400 MH_Z spectrometer. Bruker DMX-500 spectrophotometer with tetramethylsilane (TMS) as internal standard at the Central Laboratory, Jouf University. Spectra were recorded in deuterated dimethyl sulphoxide (DMSO-d_6). Splitting patterns are designated as: s: singlet; d: doublet; t: triplet; q: quartet; m: multiplet

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3-(1-(2-Phenylhydrazono) ethyl)-*1H*-indole 2 [7]**.**

A mixture of 3-acetyl indole **1** (1.59g, 1 mol), phenylhydrazine (1.08g, 1mol) and *p*-toluene sulphanilic acid (1.72g, 1 mol) in absolute ethanol (25 ml) was heated under reflux for 4hrs,.After cooling reaction mixture poured onto ice -water the brown solid will formed, filtrated and recrystallization from ethanol to give residue 2 as yellow color. Yield 93%. m.p 168-170 lit 165-167 °C. FT-IR (KBr, υ max/ cm^{-1}): 3143 (NH), 1624 (C=N), 1577 (C=C).

2-(*1H*-indol-3-yl)-2-methyl-3-(phenylamino) thiazolidin-4-one (3)

Method A

A mixture of hydrazone **2** (0.81 g, 0.002 mol), thioglycolic acid (0.024 g, 0.002 mol) and *p*-toluene sulphanilic acid (2g) in an absolute ethanol (25 mL) was added and the reaction mixture refluxed for 8 hrs. The completion of reaction was monitored by TLC. The reaction mixture after cooling poured into ice-water. The residue was filtered, recrystallized from ethyl alcohol to yield crystalline yellow.

Method B.

A mixture of 3-acetyl indole **1** (1.59 g, 1 mol), phenylhydrazine (1.08g, 1mol) and thioglycolic acid (0.92g, 1 mol) *p*-toluene sulphanilic acid (1.72 g, 1 mol) in absolute ethanol (25 ml) was heated under reflux for 4hrs,.After cooling reaction mixture poured onto ice -water the brown solid will formed, filtrated and recrystallization from ethanol. Yield 73%. m.p 205-207°C. FT-IR (KBr, υ max/ cm^{-1}): 3120 (NH), 1612 (C=O), 1558 (C=C). ^{1}H-NMR (DMSO-*d6* ppm): δ 11.92 (s, 1H, NH indole), 10.65 (s, 1H, NHPh), 8.26 (s, 1H, indole proton), 7.12-7.43 (m, 9H, Ar-H), 3.51 (dd, 2H, *J*=14, 2.5 Hz, CH_2), 2.40 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6 ppm): δ 176.96 (C=O), 137.16, 134.93, 125.79, 123.32, 122.82, 117.28, 112.30, 40.31 (CH_2), 23.48 (CH_3). Anal. calcd for: **(**$C_{18}H_{17}N_3OS$, 323.41): C, 66.85; H, 5.30; N, 12.99; S, 9.91. Found: C, 66.82; H, 5.33; N, 12.96; S, 9.89.

2-(*1H*-Indol-3-yl)-2-methyl-3-(phenylamino) imidazolidin-4-one (4)

A mixture of hydrazone **2** (2.49 g, 0.01 mol) and glycine (0.01 mol) was heated under reflux in toluene (25 ml) for 8 hr. after complete the reaction mixture then, cooled and the product solid was filtered off, dried and recrystallized from ethanol/water to afford the compound (4)

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Yield 79 %. m.p 179-181°C. FT-IR (KBr, υ max/ cm^{-1}): 3142 (NH), 1672 (C=O), 1610 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6 ppm): 1.79 (s, 3H, CH_3), 3.35-3.37 (dd, 2H, J= Hz, CH_2), 6.93-7. 37 (m, 9H, Ar-H), 7.63 (s, 1H, NH, imidazole), 8.12 (s, 1H, indole proton), 9.98 (s, 1H, NHPh), 11.23 (s, 1H, NH, indole). Anal. calcd for: ($C_{18}H_{18}N_4O$, 306.37): C, 70.57; H, 5.92; N, 18.29; Found: C, 70.59; H, 5.95; N, 18.26.

5-Benzylidene-2-(*1H*-indol-3-yl)-2-methyl-3- (phenylamino) thiazolidin-4-one (5)

A mixture of compound **3** (3.23 g, 0.01 mole) and benzaldehyde (1.06 g, 0.01 mole) was heated under reflux in absolute ethanol (30 mL) and 10% sodium hydroxide for 6 hr. After cooling the residue was filtered, washed, dried and recrystallized from ethanol. Yield 81%. m.p 217-219°C. FT-IR (KBr, υ max/ cm^{-1}): 3122 (NH), 1608 (C=O conjugated with C=C). ^{1}H-NMR (400 MHz, DMSO-d_6 ppm): 11.80 (s, 1H, NH, indole), 9.97 (s, IH, NH-Ph), 8.26 (s, 1H, indole), 1.46 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6 ppm): 27.8 (CH3), 76.9 (thiazole C-5), 112.3, 113.4, 119.7, 121.7, 123.1, 129.3, 127.4, 137.5 (indole ring), 125.8 (C=*CH*), 138.6 (thiazole C-2), 166.5 (C=O). Anal. calcd for: ($C_{25}H_{21}N_3OS$, 411.52): C, 72.97; H, 5.14; N, 10.21; S, 7.79. Found: C, 72.95; H, 5.12; N, 10.23; S, 7.82.

1-(*1H*-Indol-3-yl)ethanone hydrazine (6)[8].

A mixture of 3-acetyl indole **1** (1.59 g, 1 mol) and hydrazine hydrate (1mol) and *p*-toluene sulphanilic acid (1 mol) in absolute ethanol (25 ml) was heated under reflux for 4hrs, in presence of *p*-toluene sulphonic acid as a catalyst. After cooling reaction mixture poured onto ice -water the yellow solid will formed, filtrated and recrystallization from ethanol. FT-IR (KBr, υ max/ cm^{-1}): 3342 (NH_2), 1631(C=N) and 1568 (C=C).

3-(1-((Thiophen-2-ylmethylene)hydrazono)ethyl)-*1H*-indole (7)

A mixture of hydrazone **6** (1.73 g, 1mol) and thiophene-aldehyde (1.12 g, 1mol) in absolute ethanol (25 ml) was heated under reflux in presence of drop wise of glacial acetic acid for 5hr. After concentration the solid was formed to give yellow color, filtration and recrystallization from ethanol to give **7**. Yield 83%. m.p 198-202°C. FT-IR (KBr, υ max/ cm^{-1}): 3122 (NH), 1623 (C=N), 1546 C=C). ^{1}H-NMR (400 MHz, DMSO-d_6 ppm): 2.45 (s, 3H, CH_3), 7.03-8.12 (m, 7H, Ar-H), 8.24 (s, 1H, indole proton), 8.78 (s, 1H, N=CH). ^{13}C-NMR (100 MHz, DMSO-d_6 ppm): 22.1 (CH_3), 137.1, 134.9, 125.8, 123.2, 122.1, 121.8, 117.3, 112.6 (aromatic carbons

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of indole ring), 126.5, 127.3, 128.6, 143.2 (thiophene ring), 139.6 (N=CH), 162.1 (C=N). Anal. calcd for: ($C_{15}H_{13}N_3S$, 267.35): C, 67.39; H, 4.90; N, 15.72; S, 11.99. Found: C, 67.41; H, 4.88; N, 15.70; S, 11.97.

3-(1-(*1H*-indol-3-yl)ethylidene)hydrazono)indolin-2-one (8)

A mixture of compound **6** (0.01 mol) and *1H*-indole -2.3-dione (1.47 g, 0.01 mol) in absolute ethanol (2 ml) refluxed for 4hr in presence of *p*-toluene sulphanilic acid (1 mol). The orange residue was formed after cooling the mixture filtrated and recrystallization from ethanol. Yield 68%. m.p 224-226°C. FT-IR (KBr, υ max/ cm^{-1}): 3156 (NH), 1675 (C=O), 1608 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6 ppm): 2.92 (s, 1H, CH_3), 6.83-7.32 (m, 8H, Ar-H), 8.43 (s, 1H, CH $_{(indole)}$), 11.87 (s, 1H, NH $_{(indole)}$ exachangeable with D_2O), 10.32 (s, 1H, NH $_{(indoline)}$ exachangeable with D_2O). Anal. Calcd for ($C_{18}H_{14}N_7O$, 344.36): C, 62.78; H, 4.10; N, 28.47. Found: C, 62.81; H, 4.13; N, 28.43.

1,2 Bis(1-(1H-indol-3-yl)ethylidene)hydrazine (9)

Compounds **6** (0.01 mol) was added to 3-acetyl indole **1** (0.1 mol) in absolute ethanol (25 ml). Compound **9** has been synthesized as the same methods in compound **8**. Yield 71%. m.p 234-236°C. FT-IR (KBr, υ max/ cm^{-1}): 3196 (NH), 1610 (C=N), 1577, (C=C). ^{1}H-NMR (400 MHz, DMSO-d_6 ppm): 2.73 (s, 6H, $2CH_3$), 7.13-8.03 (m, 8H, Ar-H), 8.55 (s, 1H, CH $_{(indole)}$), 11.92 (s, 2H, NH exchangeable with D_2O). ^{13}C-NMR (100 MHz, DMSO-d_6 ppm): 22.3 ($2CH_3$), 160.3 (2C=N), 137.3, 135.2, 125.9, 123.0, 122.3, 121.6, 117.1, 112.7 (indole ring). Anal. Calcd for: (C20H18N4, 314.39): C, 76.41; H, 5.77; N, 17.82. Found: C, 76.39; H, 5.75; N, 17.85.

3-Acetylindole thiosemicarbazone (10) [9]**.**

A mixture of 3-acetyl indole **1** (1.59 g ,1 mol), thiosemicarbazide (0.01 mol) and *p*-toluene suphonic acid (0.01 mol) in ethanol (25 ml) was heated under reflux for 4 hr. after cooling the solid was formed filtration and recrystallization by ethanol. Yield 87%. FT-IR (KBr, υ max/ cm^{-1}): 3419 (NH_2), 3143 (NH), 1610 (C=N), 1577, (C=N), 1242 (C=S).

5-(*1H*-Indol-3-yl)-5-methyl-4, 5-dihydro-1, 3, 4-thiadiazol-2-amine (11)

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Thiosemicarbazide derivative **10** (3.4 mol) dissolved in ethanol containing of concentrated hydrochloric acid (0.5 ml), the reaction mixture was heated under reflux for 4hr. After cooling and dilution with water, the precipitated product was formed and filtered, washed with water, dried and recrystallized ethanol to give **11** as white- green powder. Yield 70%. m.p 207-209 °C. FT-IR (KBr, υ max/ cm^{-1}): 3373 (NH_2), 3156 (NH), 1612 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6 ppm): 2.38 (s, 3H, CH_3), 7.13-7.44 (m, 4H, Ar-H), 7.36 (s, 1H, indole ring), 8.17 (s, 2H, NH_2), 8.27 (s, 1H, NH thiadiazole ring), 11.83 (s, 1H, NH indole ring).

_N_-(4-Acetyl-5-(_1H_-indol-3-yl)-5-methyl-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (12)

A mixture of thiosemicarbazone derivative **10** (3.45g, 0.01mol) and the acetic anhydride (10 ml) was heated under reflux for 7h with stirring after complete reaction by TLC and after cooling, the reaction mixture was poured onto ice-cold water with stirring. The formed residue was filtered, dried and recrystallized from ethanol and DMF (2:1) to give **12.** Yield 69%. FT-IR (KBr, υ max/ cm-1): 3128 (NH), 1653, 1707 (C=O), 1541(C=C).^{1}H-NMR (400 MHz, DMSO-d_6 ppm): 11.83 (s, 1H, $NHCOCH_3$). 2.72 (s, 3H, CH_3), 2.42 (s, 3H, $NCOCH_3$), 1.64 (s, 3H, $NHCOCH_3$), 8.87 (s, 1H, indole ring), 7.32-8.31 (m, 4H, Ar-H), 9.66 (s, 1H, NH indole ring), 11.88 (s, 1H, $NHCOCH_3$).^{13}C-NMR (100 MHz, DMSO-d_6 ppm): 174.8 ($NHCOCH_3$), 169.0 ($NCOCH_3$), 149.8 (N=*C*-S), 28.8 ($NCOCH_3$), 24.4 ($NHCOCH_3$).

5-(_1H_-indol-3-yl)-5-methyl-4,5-dihydro-_1H_-1,2,4-triazole-3-thiol (13)

A mixture of thiocarbothioamide derivative **10** (0.005mol) was heated under reflux for 6 h in the presence of piperidine (2ml) and ethanol (25ml). The reaction mixture was poured onto ice-cold water and the reaction mixture was neutralized with acetic acid (20%). The solid obtained was filtered, dried and crystallized from solvent (acetone and ethanol respectively) to give **13**. Yield 63%. FT-IR (KBr, υ max/ cm^{-1}): 3125 (NH), 1612 (C=N).^{1}H-NMR (400 MHz, DMSO-d_6 ppm): 2.32 (s, 3H, CH3), 6.98-7.42 (m, 4H, Ar-H), 8.23 (s, 1H, indole ring), 9.32(s, 1H, NH triazole), 11.79 (s, 1H, NH, indile ring). ^{13}C-NMR (100 MHz, DMSO-d_6 ppm): 32.2 (CH_3), 137.2, 134.8, 125.7, 123.3, 122.1, 121.7, 117.1, 112.5 (aromatic carbons of indole ring), 149.6 (C=N).

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2-(1-(*1H*-Indol-3-yl)ethylidene)-*N*-(-thiophen-2-ylmethylene)hydrazine-1-carbothioamide (14)

A mixture of thiophene-carboxaldehyde (0.005 mol) and thiocarbothioamide derivative **10** (0.005mol) was heated under reflux for 2 h in presence of acetic acid. The formed precipitate was filtered, washed with water, dried and crystallized from the appropriate solvent to yield **14.** Yield 81%. FT-IR (KBr, υ max/ cm^{-1}): 3127 (NH), 1610(C=N), 1525 (C=), 1242 (C=S).^{1}H-NMR (400 MHz**,** DMSO-d6 ppm):10.07 (s, 1H, NH-C=S), 11.97 (s, 1H, NH indole), 8.82 (s, 1H, N=CH), 8.27 (s, 1H, indole ring), 6.83-8.26 (m, 7H, Ar-H), 2.46 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6 ppm): 193.1 (C=S), 170.8 (CH_3-***C***=N-), 156.3 (N=*C*H), 138.1, 137.1, 134.9, 131.5, 131.1, 129.3, 128.8, 125.7, 123.2, 122.1, 121.8, 117.2, 112.6, 27.7 (CH_3). Anal. Calcd for: ($C_{16}H_{14}N_4S_2$; 326.44): C, 58.87; H, 4.32; N, 17.16; S, 19.64.

1-(*1H*-indol-3-yl)-*N*-(4-nitrophenyl) ethan-1-imine (15)

3-Acetylindole **1** (1.59 g, 0.01 mol) was dissolved in 20 ml of absolute ethanol, p-nitro benzaldehyde (0.01 mol) was then added, 2 drops of glacial acetic acid. The reaction mixture was refluxed for 5hr, the mixture was cooled, poured onto ice-water, and the solid product was filtered off and recrystallized from ethanol to give **15.** Yield 78%. FT-IR (KBr, υ max/ cm^{-1}): 3155 (NH), 1627 (C=N), 1597 (C=C), 1315-1442 (-NO_2).

2-(*1H*-indol-3-yl)-2-methyl-3-(4-nitrophenyl) thiazolidin-4-one (16)

A mixture of Schiff base **15** (1.59 g, 0.01 mol) with thioglycollic acid (0.92 g, 0.01 mol) in dry toluene (25 ml). The reaction mixture was refluxed for 6 hr., cooled and recrystallization from ethanol. Yield 65%. FT-IR (KBr, υ max/ cm^{-1}): 1635 (C=O), 1313-1512 (NO_2). 1.97 (s, 3H, CH_3), 3.69-3.78 (dd, 2H, *J*= 8 Hz, CH_2), 6.79 (d, 2H, *J*= 8.4 nitro phenyl), 8.01 (d, 2H, *J*= 8.4 Hz, nitro phenyl), 7.43-8.12 (m, 4H, Ar-H), 8.56 (s, IH, indole proton), 10.23 (s, 1H, NH). Anal. Calcd for: ($C_{18}H_{15}N_3O_3S$; 353.40): C, 61.18; H, 4.28; N, 11.89; S, 9.07. Found: C, 61.10; H, 4.24; N, 11.84; S, 9.13.

***N, N'*-(Ethane-1,2-diyl)bis(1-(*1H*-indol-3-yl)ethan-1-imine (17)**

The reaction of 3-acetylindole **1** (6.36 g, 4 mol) and ethylenediamine (1.2 g, 2 mol) in absolute ethanol (30 ml) was heated under reflux for 3 hrs. The solid was collected after cooling. After

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evaporating the solvent under vacuum a yellow solid formed recrystallization by ethanol to give **17.** Yield 79 %. m.p 239-241°C. FT-IR (KBr, υ max/ cm^{-1}): 3216 (NH), 1623 (C=N).^{13}C-NMR (100 MHz, DMSO-d_6 ppm): δ24.3 (2CH3), 163.6 (2C=N) and 63.1(2CH_2). Anal. Calcd for: ($C_{22}H_{22}N_4$; 342.45): C, 77.16; H, 6.48; N, 16.36. Found: C, 77.01; H, 6.83; N, 16.31.

Biological activity

Antibacterial Activity

Microorganisms

Clinical isolates of *Staphylococcus aureus* (*S.aureus*), *Bacillus cereus* (*B.cereus*), *Klebsiella Pneumoniae* (*K.pneumonia*), *Escherichia coli* (*E.coli*), and *Pseudomonas aeruginosa* (*P.aeruginosa*) were used in this study. Isolates were identified based on their morphology and biochemical characteristics [12].

Media, Chemicals, and antimicrobial agents

All media were prepared according to the manufacturer's instructions and pH was adjusted at 7.4. Media were sterilized by autoclaving at 121˚C for 15 min. unless otherwise mentioned.

-Nutrient agar

- Nutrient broth
- Mannitol salt agar
- MacConkey agar
- Cetrimide agar
- Eosin methylene blue.

Antimicrobial agents and chemicals

High purity Dimethylformamide (DMF) and Dimethyl sulfoxide were used as solvents. Gentamicin (80 mg/2 ml, EIPICO Co., Egypt) was used as standard antibacterial agent.

Tools and instruments

The following tools and instruments were used throughout the study: sterile disposable Petri dishes, sterile swabs, Eppendorf tubes, blue and yellow tips, Wassermann tubes, micropipettes, Vernier caliper, laminar airflow, incubator, autoclave, and hot air oven.

Methods

Identification of bacterial isolates

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Clinical isolates of *S.aureus*, *B.cereus*, *K.pneumonia*, *E.coli* and *P.aeruginosa* were identified according to standard microbiological methods. Cultures were subjected to gram staining before performance of some biochemical tests to confirm identity. Identified isolates were then grown on nutrient agar slant and kept at 4℃ till use.

Screening of the antibacterial activity of synthesized compounds

The gram positive bacteria *S.aureus* and *Bacillus cereus* and the gram negative bacteria *E.coli, K.pneumonia,* and *P.aeruginosa* were used in this study. The antibacterial activity of synthesized compounds was evaluated using the agar well diffusion method. Bacterial isolates were grown overnight at 37℃, diluted in nutrient broth medium to 0.5 MacFarland standard. Then 100 µl of diluted cultures were inoculated into 50 ml of nutrient agar at 50 ℃, mixed well, poured into 10 cm Petri dishes, and left to solidify. Wells in the agar plates were made by Wassermann tubes sterilized in ethanol. A volume of one hundred microliter of the tested compounds (5mg/ml) was applied in the well and plates were left for pre-diffusion before overnight incubation at 37℃. Positive Control wells of gentamicin (1 mg/ml) were included in each experiment. DMSO and DMF were used as solvents for tested compounds and were included also as negative controls. The inhibition zone diameter was measured in mm using Vernier caliper. The inhibition zone diameters of used solvents were subtracted from those correspond to each of tested compounds. Activity index was calculated using the following formula

Activity index= inhibition zone of test compound / inhibition zone of standard

In vivo antioxidant & anti-inflammatory screening

Sigma-Aldrich Chemicals provided the majority of the chemicals used (St. Louis, MO). Seventy mature albino male rats weighing 150–200 g were obtained from the Tanta University Animal House for this investigations. The rats were kept in wire mesh cages at room temperature, where they were fed a standard rat food and had full access to water. The trials were carried out in accordance with rules of the Animal Care guidelines.

Experimental design and animal grouping: Male albino rats were divided into (10 rats for each).

Group I: control group received 1 ml solvent control composed of 2% Carboxymethyl cellulose

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(CMC)) taken per oral.• Group II: Inflammatory group in which Inflammation was induced in the sub-planter tissue of the left hind paw in each rat after 60 minutes of injection of 0.1 ml carrageenan of (1% in saline) into the sub-planter tissue. [13]. Group III: Treated inflammatory group.

Subgroup (A) received test compounds (**4**) (50 mg/kg Bodyweight, per oral) suspended in 2% CMC. Then, inflammation was induced in the sub-planter tissue of the left hind paw in each rat after 60 minutes of injection of 0.1 ml carrageenan of (1% in saline) into the sub-planter tissue. (1). Subgroup (B) received test compounds (**14**) (50 mg/kg Bodyweight, per oral) suspended in 2% CMC. Then, inflammation was induced as in subgroup A. Subgroup (C) received test compounds (**8**) (50 mg/kg Bodyweight, per oral) suspended in 2% CMC. Then, inflammation was induced as in subgroup A. Subgroup (D) received test compounds (**13**) (50 mg/kg Bodyweight, per oral) suspended in 2% CMC. Then, inflammation was induced as in subgroup A. Subgroup (E) received test compounds (**7**) (50 mg/kg Bodyweight, per oral) suspended in 2% CMC. Then, inflammation was induced as in subgroup A.

A sample of blood was taken. Rats were sacrified after anesthesia, and blood samples were collected into sterile centrifuge tubes, which were allowed to clot for 30 minutes at room temperature before being spun at 5000 rpm for 10 minutes. Sera were gathered and kept at 70°C until needed.

The following tests were applied to all groups:

A-Using an automated analyzer (Hitachi Ltd.), the following parameters were assessed:

Oxidative stress parameter:

Serum Malondialdehyde (MDA) levels are measured using a spectrophotometric method based on the color produced at 532 nm during the reaction of MDA with thiobarbituric acid [14]

Antioxidant enzyme activity:
Serum catalase (CAT) activity was determined using a colorimetric kit (CAT. No. CA 25 17) provided by the BIODIAGNOSTIC Company in Egypt.

The total antioxidant capacity (TAC) of serum was determined using a kit provided by the BIODIAGNOSTIC Company in Egypt, according to the method of Koracevic et al. [15].

Renal toxicity was assessed using a commercial kit from Egypt's BIODIAGNOSTIC Company for serum creatinine and blood urea nitrogen levels (BUN).

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Liver toxicity was determined by measuring serum aspartate transaminase (AST) and alanine transaminase (ALT) activity with commercially available kits from the BIODIAGNOSTIC Company in Egypt.

B- Level of NF-B (an inflammatory mediator) in the blood. It was tested with an ELISA kit, according to the manufacturer's pro (EIAab Science Co., Wuhan, China).

Statistical Analysis:

The entire data was presented as means ± standard error (SE). The statistical significance was calculated using SPSS 18.0 software. One-way ANOVA analysis was used to determine significance difference between studied groups. For comparison of the means, Turkey's significant difference test was performed. Statistically significant was considered when p Values were ≤ 05.

Molecular Docking (MD)

Molecular docking (MD) or docking studies are the computational strategies to understand the nature of synthesized compounds inside protein receptors and to distinguish the way by which they induced fit within the pocket. In this study, we manipulate the associations of compound **5, 8** and **11** with *Staphylococcus aureus (S. aureus), Bacillus cereus (B. cereus), Escherichia coli (E. coli), Klebsiella pneumonia (K. pneumoniae) and Plasmodium aeroginosa (P. aeroginosa).* Auto dock Vina 1.1.2 with PyRx was utilized that has the Lamarckian genetic algorithm (LGA) along with empirical energy free function for scoring and conducts the practice efficiently. Dockings runs were achieved by taking the grid box with dimensions in Angstrom X: 51.12, Y: 52.87, Z: 47.25. Further energy minimization parameters were set with force field *Uff* and optimization algorithm as conjugate gradients. To achieve the results, crystal structure of *Staphylococcus aureus* having PDB ID: 1VQQ, *Bacillus cereus* having PDB ID: 1BVT, *Escherichia coli* glutathione transferase protein having PDB ID: 5HFK, *Klebsiella pneumonia having PDB ID: 2OV5* and *Plasmodium aeroginosa* proteins having PDB ID: 3SZT were downloaded from protein data bank (RCSB) and saved in Pdb format. These targets were purified with removal of water molecules and co crystallized ligand. Afterwards targets were added with polar hydrogen and saved as purified receptors. Synthesized compounds **5, 8** and **11** were sketched on latest version of professional Chemdraw 20 software and stored as Pdb file after addition of polar hydrogen. Energy minimization is a compulsory task to achieve as it make the interaction more realistic.

Visualization of results was carried out through Bio via discovery studio visualizer DSV and ligplot + software.

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