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TRANSFORMATION OF BENZOXAZINONE DERIVATIVES TO SOME INTERESTING HETEROCYCLIC COMPOUNDS WITH EXPECTED BIOLOGICAL ACTIVITY

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Abstract – The newly synthesized iodobenzoxazinone derivative was reacted with benzoyl hydrazide, sodium azide, hydrazine hydrate, *p*-toluidine, hydroxylamine hydrochloride and formamide to give (quinazoliny)benzamide, (oxazinyl)benzohydrazide, imidazolecarboxamide, (tetrazolyl)benzoic acid, 3-aminoquinazolinone, *p*-toylquinazolinone, hydroxyquinazolinone and quinazolinone derivatives respectively. Reaction of hydroxyquinazolinone with acetic anhydride and ethyl chloroacetate afforded (quinazoliny)acetate and ethyl (quinazolinyloxy)acetate derivatives. The quinazolinone reacted with benzoyl chloride, acetyl chloride and ethyl chloroacetate to afford *N*-(3-benzoyl-dihydroquinazoliny)-*N*-phenylbenzamide, 3-acetylquinazolinone and ethyl quinazolinyacetate respectively. The acetohydrazide which was synthesized from the reaction of the ethyl quinazoliny acetate with hydrazine hydrate was used as a starting material for preparation of some other quinazolinone derivatives. The newly synthesized compounds were characterized by spectroscopic tools and some of them were screened for antibacterial and antifungal activity.

Benzoxazinone derivatives are considered to be important chemical synthons of various physiological

significances and pharmaceutical utilities. They possess a variety of biological effects including anti-tubercular,¹ antifungal, antimalarial, anticancer, anti-HIV antiviral and antibacterial activities.^{2,3}

Quinazolinones are compounds with wide spectrum of biological activities, including: anticancer, anticonvulsant, anti-inflammatory, anti-tubercular and antibacterial effects.⁴⁻¹¹

Iodine was selected because it has received considerable attention in organic synthesis¹¹ due to its high tolerance to air and moisture, low-cost, nontoxic nature and ready availability. Presence of iodine increases the lipophilicity of the molecules. Iodine increases lipophilicity of the compound more than fluorine, chlorine and bromine as iodine is bigger and processes higher polarizability. Iodine atom can change considerably the pharmacology of the original molecule due to: (i) a change of the torsion angle; (ii) a change of the electronic density and (iii) the increase of lipophilicity.

The anthranilic acid (2-aminobenzoic acid) has two different functional groups (-CO₂H and -NH₂) and therefore, it is often used in the synthesis of heterocyclic compounds as it can readily undergo condensation and nucleophilic reactions.

Considering the special structure of benzoxazinone derivatives, two sites are available for nucleophilic attack, that is, two different sites with partial positive charges that can lead to the opening of heterocyclic part of benzoxazinone derivatives by different attacking nucleophiles. In most of cases, re-closure of the heterocyclic part of the molecule provides a new compound with interesting chemical properties.¹²

From all the above factors we ensue to synthesis novel benzoxazinone derivatives containing iodine atom, and also the present investigation is a continuation of our earlier study on benzoxazinone¹³ and quinazolinone derivatives.^{14,15} We report herein the synthesis of new biological active compounds based on benzoxazinone nucleus and evaluate their antimicrobial activity.

Three target compounds were prepared; the first included the benzoxazinone derivative (**4**), the second consisted of the phenylaminoquinazolinone derivative (**14**), and the third consisted of the quinazolinyl acetohydrazide derivative (**18**). The dynamic benzoxazinone derivative (**4**) was prepared from the reaction of 5-iodoanthranilic acid (**1**) with phenyl isocyanate (**2**) in dioxane to give the (phenylureido)benzoic acid derivative (**3**) followed by reflux in acetic anhydride. These results are depicted in Scheme 1. The IR spectrum of (phenylureido)benzoic acid derivative (**3**) revealed bands at

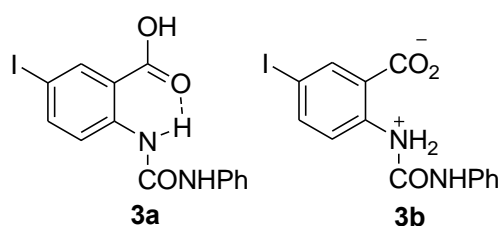
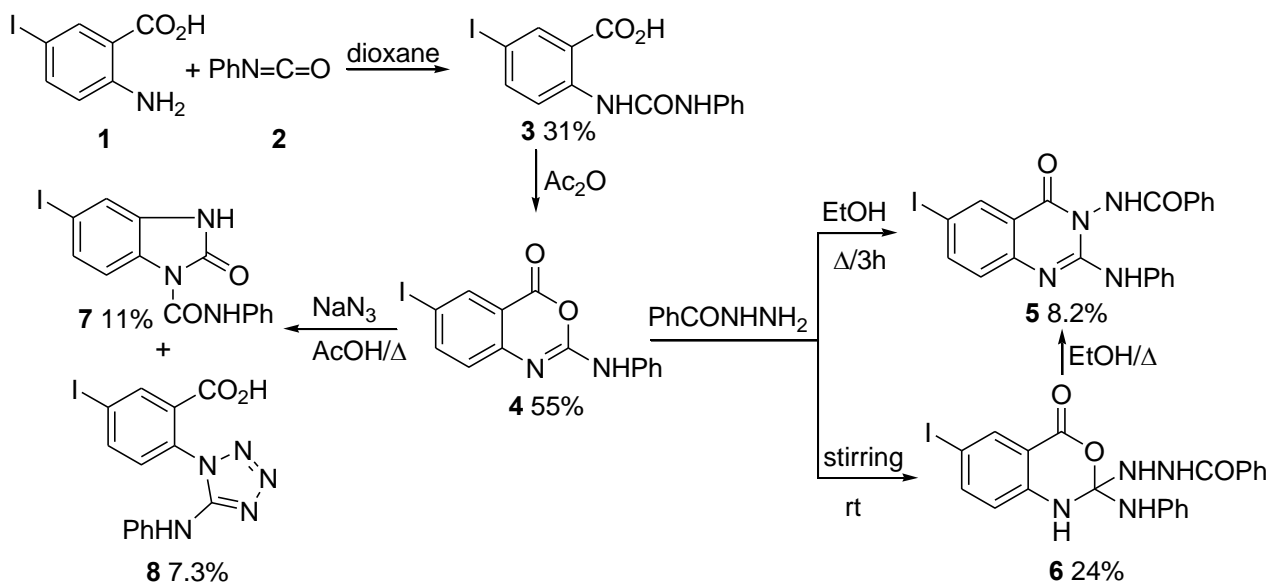


Figure 1

3314 and 3299 cm^{-1} for OH and NH respectively, and also bands at 1665, 1596 and 1383 cm^{-1} attributable to C=O, ionized carboxyl group $-\text{CO}_2^-$ and bending vibration of $-\text{NH}_3^+$ respectively. The lowering of the C=O value is due to the formation of hydrogen bond (**3a**) or the formation of the carboxylate anion (**3b**). These results are depicted in Figure 1.

The first group of compounds was prepared as shown in Schemes 1 and 2. The reaction of benzoxazinone derivative (**4**) with benzoyl hydrazide depended on the reaction conditions. Bond breaking needs more energy than the addition on the double bond, therefore, the reflux of the benzoxazinone derivative (**4**) with benzoyl hydrazide in ethanol afforded the more stable (quinazoliny)benzamide derivative (**5**). On the other hand its stirring with the benzoyl hydrazide at room temperature afforded the (oxazinyl)benzohydrazide derivative (**6**). Heating the (oxazinyl)benzohydrazide derivative (**6**) in EtOH afforded the (quinazoliny)benzamide derivative (**5**).

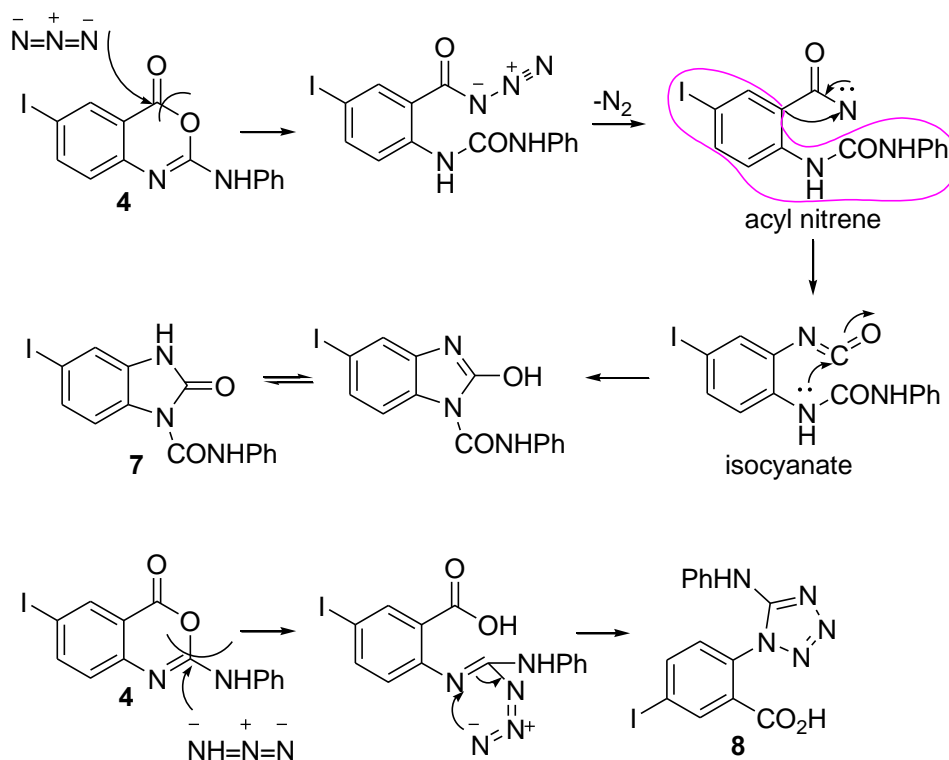


Scheme 1

Benzoxazinone derivative (**4**) reacted with sodium azide in boiling acetic acid to give a mixture of the corresponding imidazolecarboxamide (**7**) and tetrazolylbenzoic acid (**8**) derivatives. The reaction of the benzoxazinone derivative (**4**) with sodium azide and rearrangement via nitrene and isocyanate intermediates afforded the imidazolecarboxamide derivative (**7**) (Scheme 1). The mechanism of formation of the imidazolecarboxamide derivative (**7**) consists of the release of nitrogen gas followed by an aryl shift of the aryl group from the carbonyl carbon to the closest nitrogen. The release of gas drives the reaction forward and results in the formation of the isocyanate product via nitrene intermediate which can potentially react further with the nitrogen nucleophile (Curtius rearrangement). While the benzoic acid derivative (**8**) was formed through nucleophilic attack followed by ring opening and ring closure (Scheme

2). Treatment of the benzoxazinone derivative (**4**) with hydrazine hydrate, *p*-toluidine and hydroxylamine hydrochloride afforded 3-aminoquinazolinone, *p*-toylquinazolinone, and hydroxyquinazolinone derivatives (**9-11**) respectively (Scheme 3).

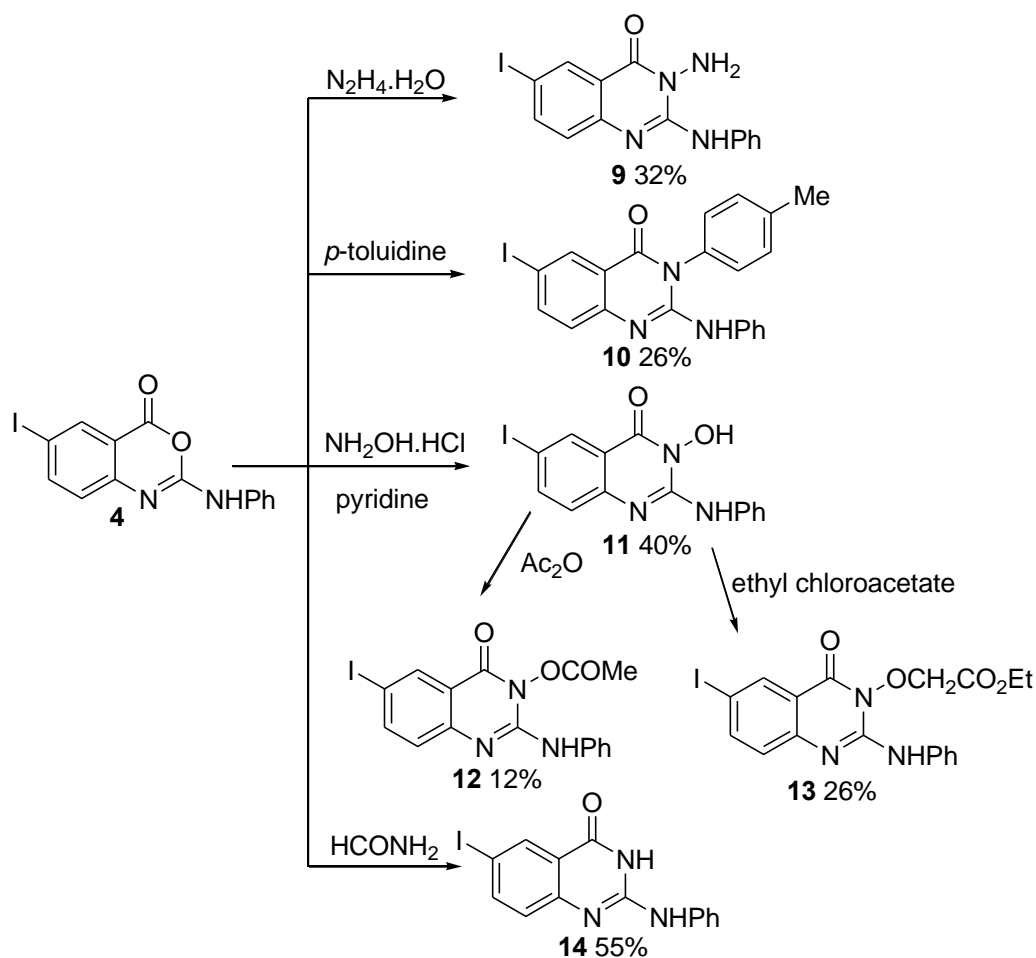
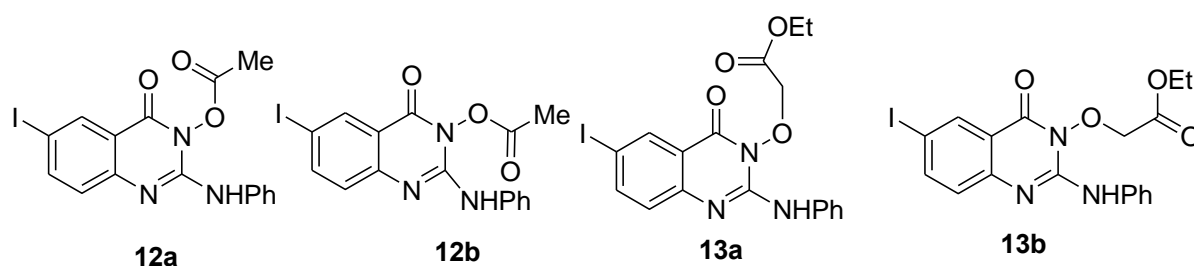
The formation of 3-aminoquinazolinone derivative (**9**) was drawn from IR spectrum that revealed NH₂ and NH stretching bands at 3293, 3259 and 3194 cm⁻¹ respectively. While the ¹H NMR spectrum revealed the NH₂ and NH protons as singlet at 2.51 and 9.85 ppm, in addition to the other aromatic protons appearing as multiplet at 6.98–7.58 ppm. ¹H NMR spectrum of *p*-toylquinazolinone derivative (**10**) revealed the CH₃ protons as singlet at 2.24 ppm. The IR spectrum of hydroxyquinazolinone derivative (**11**) displayed bands consistent with OH, NH and C=O. further evidence is gained from ¹H NMR spectrum as it exhibited signals for OH and NH at 10.91 and 11.60 respectively.



Scheme 2

The reaction of hydroxyquinazolinone derivative (**11**) with acetic anhydride and ethyl chloroacetate to afford quinazolinylacetate and ethyl quinazolinylacetate derivatives (**12**) and (**13**) respectively confirmed the presence of hydroxyl group in hydroxyquinazolinone derivative (**11**). The IR spectra of compounds (**12**) and (**13**) devoid the presence of OH stretching band; beside the appearance of two bands

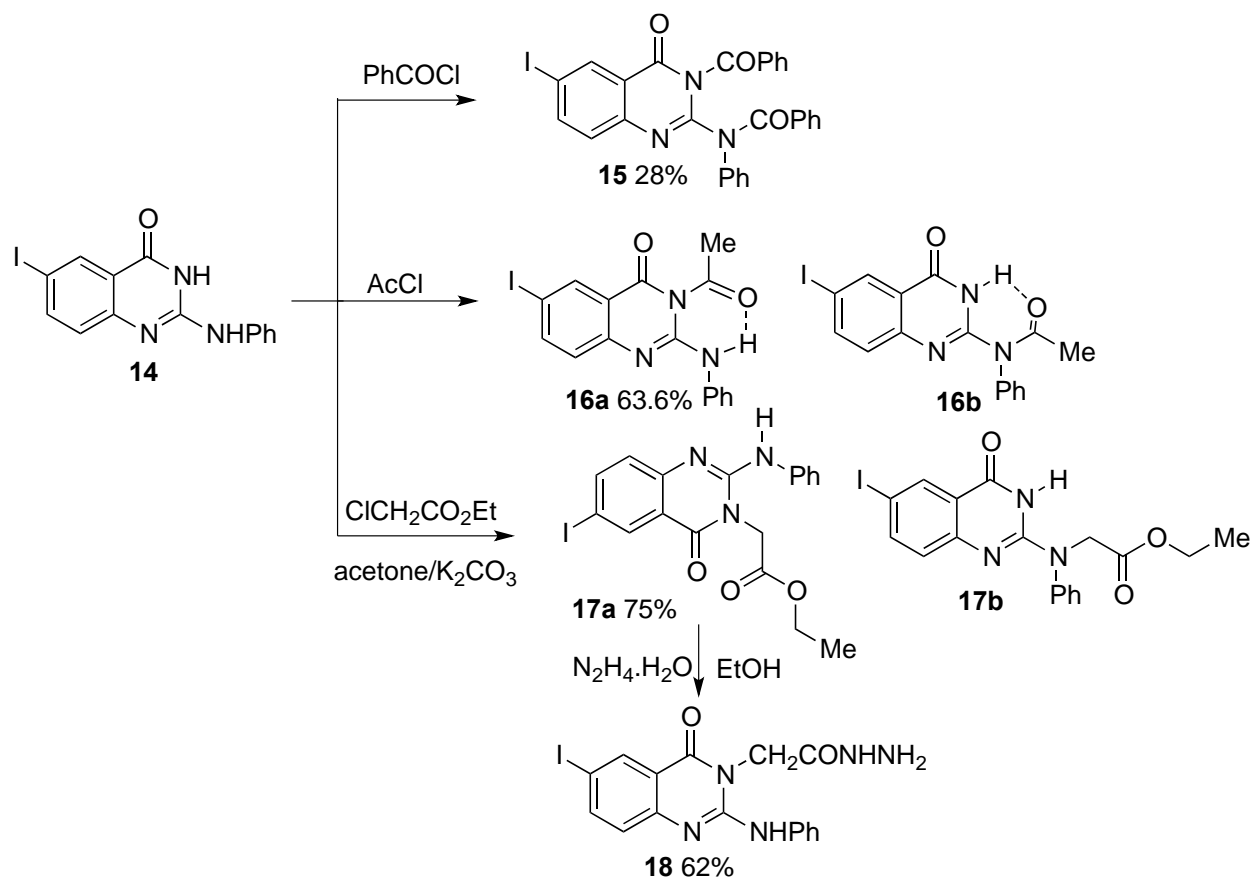
for the carbonyl group of each compound at 1734, 1715 cm^{-1} and at 1742, 1709 cm^{-1} respectively due to mutual induction (field effect) of the two carbonyl groups; the nonbonding electrons of the oxygen atoms of the two carbonyl groups undergo repulsion when they are close together in the molecule; this results in a change in the hybridization state of the oxygen atoms, and therefore a shift in C=O stretching frequency. Further support for the proposed structures of compounds **12** and **13** are gained from ^1H NMR spectra which exhibited signal for CH_3 protons at 2.12 ppm for compound **12**, and signals for CH_3 and CH_2 protons at 1.32 and 4.18 respectively for compound **13** beside signals of aromatic protons and NH.



Scheme 3

Quinazolinone derivative (**14**) was prepared through the reaction of the benzoxazinone derivative (**4**) with formamide. ^1H NMR spectrum showed signals at 2.33 and 12.38 ppm attributable to 2NH protons.

The second group of compounds was prepared as shown in Scheme 4.



Scheme 4

There are two possibilities for the electrophiles to react with nitrogen atoms of the quinazolinone derivative (**14**), either with the nitrogen in position 3 or that one in the side chain. Benzoylation was occurred on the both positions to afford the *N*-(3-benzoylquinazolinyl)-*N*-phenylbenzamide derivative (**15**); while acetylation and esterification was occurred on the nitrogen in position 3 to afford 3-acetyl-quinazolinone derivative (**16a**) and the ethyl quinazolinylacetate derivative (**17a**) not (**16b**) and (**17b**). The IR spectrum of *N*-phenylbenzamide derivative (**15**) devoid any signal for NH, and showed signal for C=O at 1693 cm^{-1} , the highly value is due to mutual induction between the two C=O groups. The structures of the 3-acetylquinazolinone derivative (**16a**) and the ethyl quinazolinylacetate derivative (**17a**) were confirmed by spectroscopic data. The IR and the ^1H NMR spectra of 3-acetylquinazolinone derivative (**16a**) showed the presence of $\nu_{\text{C=O}}$ at 1680 cm^{-1} and δ for NH at 12.30 which is in accordance with the presence of a hydrogen bond. The isomer **16b** was excluded through NOE difference experiments. Thus irradiation of

the NH proton in compound **16a** at δ 12.30 enhanced the aromatic protons and no such enhancement would be possible for the CH₃ protons. The IR spectrum of compound **17a** showed bands at 3338, 1735 and 1674 cm⁻¹ for NH and C=O for ester and the quinazolinone ring respectively. The ¹H NMR spectrum shed further light on the proposed structure as it exhibited triplet and quartet signals attributed to CH₃ and CH₂ of the ester respectively. The isomer **17b** was excluded through NOE difference experiments. Thus irradiation of the NH proton in compound **17a** at δ 12.25 enhanced the aromatic protons and no such enhancement would be possible for the CH₂ protons.

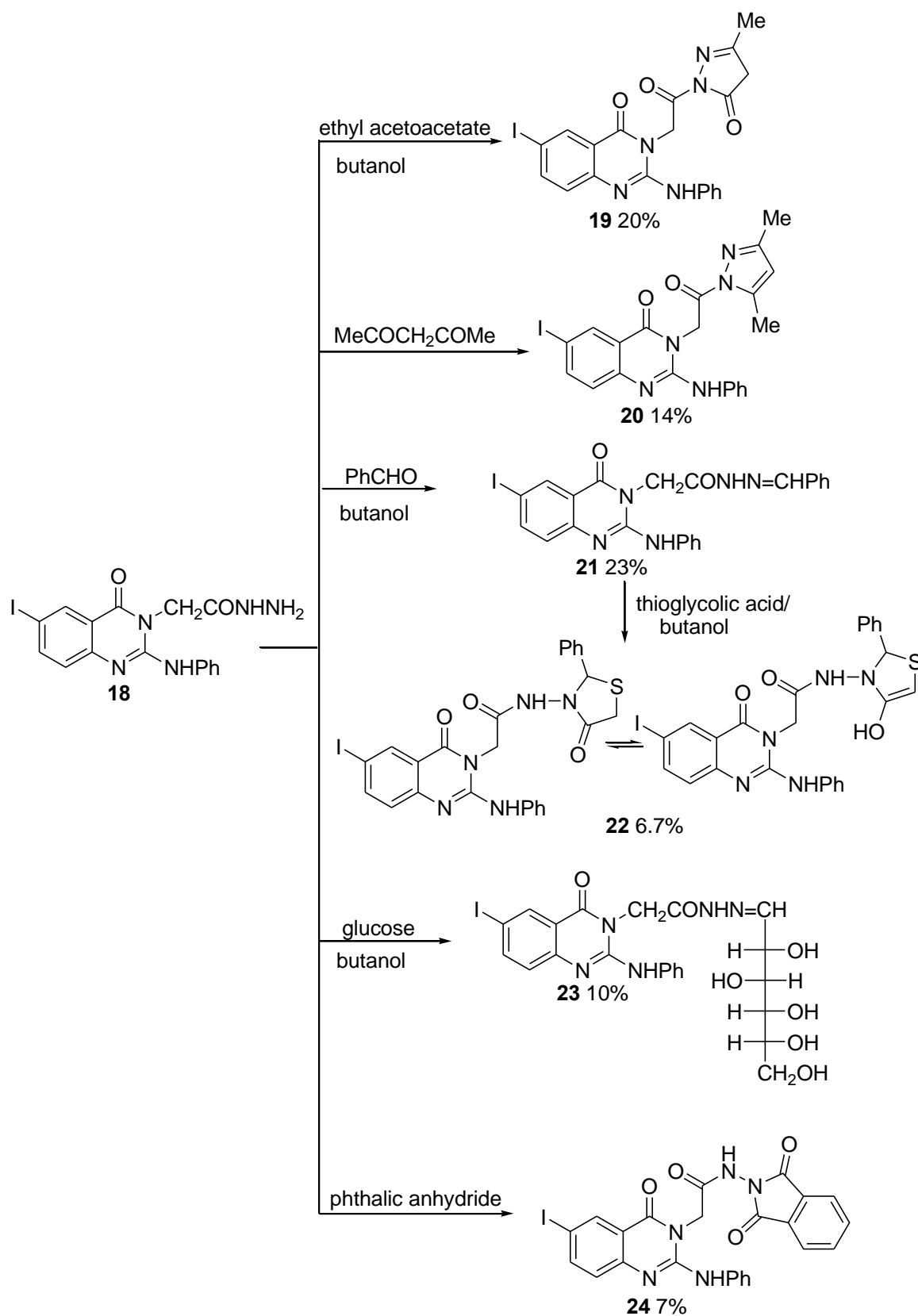
Hydrazinolysis of ethyl quinazolinylacetate derivative (**17**) with hydrazine hydrate gave the corresponding quinazolinylacetohydrazide derivative (**18**), which was used as a starting material for the preparation of some other quinazolinone derivatives. The IR spectrum revealed the presence of NH₂ group. The ¹H NMR spectrum showed signals for NH and NH₂ groups. Further evidence was gained from mass spectrum as it showed the correct molecular ion peak beside some important peaks.

The third group of compounds was prepared as shown in scheme 5. The behavior of quinazolinylacetohydrazide derivative (**18**) towards carbon electrophiles has been investigated with a view for obtaining some interesting quinazoline derivatives. Interaction of the quinazolinylacetohydrazide derivative (**18**) with ethyl acetoacetate and acetylacetone afforded the pyrazolyl derivatives (**19**) and (**20**) respectively. These results are depicted in Scheme 5. The IR and the ¹H NMR spectra of the pyrazolyl derivatives (**19**) and (**20**) devoid any signals for NH₂ group; and the presence of cyclic CH₂ and =CH respectively. The mass spectra showed the molecular ion peak M⁺ and M⁺² for the cyclic pyrazolyl derivatives (**19**) and (**20**) respectively.

On treatment of quinazolinylacetohydrazide derivative (**18**) with benzaldehyde, the *N'*-benzylidenequinazolinylacetohydrazide derivative (**21**) was obtained. Inspection of its ¹H NMR spectrum revealed the existence of singlet signal at δ 5.31 corresponding to =CH proton. Subjecting the benzylidenequinazolinylacetohydrazide derivative (**21**) to the action of thioglycolic acid afforded the thiazolidinylacetamide derivative (**22**). This reaction assumed to proceed by addition followed by cyclodehydration. The infrared spectrum of compound **22** is devoid of any absorption corresponding to OH group. Instead, it exhibited bands characteristic of C=O group. This is a good evidence for the existence of compound **22** as the keto form in the solid state. While, the presence of one exchangeable broad singlet signal at 11.83 ppm corresponding to OH proton and a signal at 5.31 ppm for =CH proton in the ¹H NMR spectrum of compound **22** supported its existence in the enol form in the liquid state. The NOE irradiation at 11.83 ppm enhanced the signal at 5.31, therefore the irradiated signal corresponds to =CHS.

(Pentahydroxyhexylidene)acetohydrazide derivative (**23**) was synthesized by the reaction of the quinazolinylacetohydrazide derivative (**18**) with glucose in butanol. The presence of a basin peak

centered at 3344 cm^{-1} in the IR spectrum indicated the presence of several OH and NH groups.



Scheme 5

Reaction of the quinazolinyllaceto-hydrazide derivative (**18**) with phthalic anhydride afforded the dioxoisindolinyl derivative (**24**). The structure of dioxoisindolinyl derivative (**24**) is confirmed by spectroscopic data. The IR spectrum showed bands at 1663 cm^{-1} due to carbonyl groups. The $^1\text{H NMR}$ displayed two singlet signals in the down field region attributable to 2NH protons; and devoid of any signals for NH_2 protons.

Biological evaluation (*in vitro* antimicrobial measurement)

Some of the newly synthesized compounds were screened for their antimicrobial activity against *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6635), *Salmonella typhimurium*, *Escherichia coli* (ATTC-25922), *Candida albicans* and *Aspergillus fumigatus* microbial strains by using disc–agar diffusion method. Antibacterial activity was determined by measuring the diameter of inhibition zone. These results are depicted in Table 1.

Table 1. Antimicrobial screening results of the tested compounds

Organism	<i>S.aureus</i>	<i>B.subtilis</i>	<i>S.typhimurium</i>	<i>E.coli</i>	<i>C.albicans</i>	<i>A.fumigatus</i>
Conc/	1 mg/mL/	1 mg/mL/	1 mg/mL/	1 mg/mL/	1 mg/mL/	1 mg/mL/
Sample no	0.5 mg/mL	0.5 mg/mL	0.5 mg/mL	0.5 mg/mL	0.5 mg/mL	0.5 mg/mL
6	-/-	-/9	-/-	11/8	30/26	-/-
10	-/-	-/-	12/9	-/-	24/18	-/-
14	-/-	-/10	-/-	-/-	24/20	-/-
15	13/9	9/9	9/17	-/-	31/27	-/-
16	-/-	-/-	-/-	-/-	-/-	-/-
18	-/-	-/-	-/-	-/-	20/15	-/-
23	-/-	-/-	-/-	-/-	15/11	-/-
24	-/-	-/-	-/-	-/-	17/15	-/-
#Standard	35/26	35/25	36/28	38/27	35/28	37/26

#Standard: chloramphenicol in the case of Gram-positive bacteria, cephalothin in the case of Gram-negative bacteria, and cycloheximidine in the case of fungi.

3,1-Benzoxazin-4-ones can be considered as semi-acid anhydrides which undergo many of the reactions of true acids anhydrides but at a slower rate. This special reactivity allows this class of compounds to be quite useful as serine protease inhibitors; inactive enzymes such as chymotrypsin,¹⁶ human leucocytes,^{17,18} porcine pancreatic elastase, cathepsin G-7 and C 1r serine protease.¹⁹

Mechanistically, the inactivation involves nucleophilic attack of the active site serine hydroxyl group on the carbonyl group of the benzoxazinone, which leads to ring opening and formation of an acylated

enzyme (inactive form). The chemical stability and potency of the benzoxazinones can be tuned by changing the substituents (R), which influence the reactivity of the carbonyl group by electronic and steric effects. These results are depicted in Figure 2.

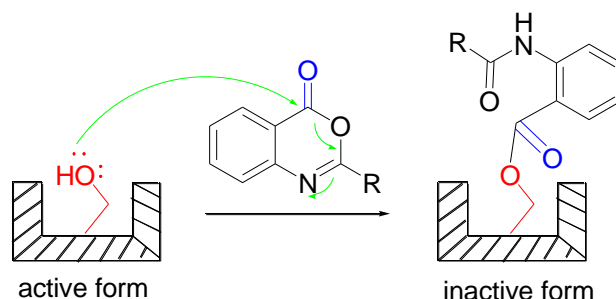
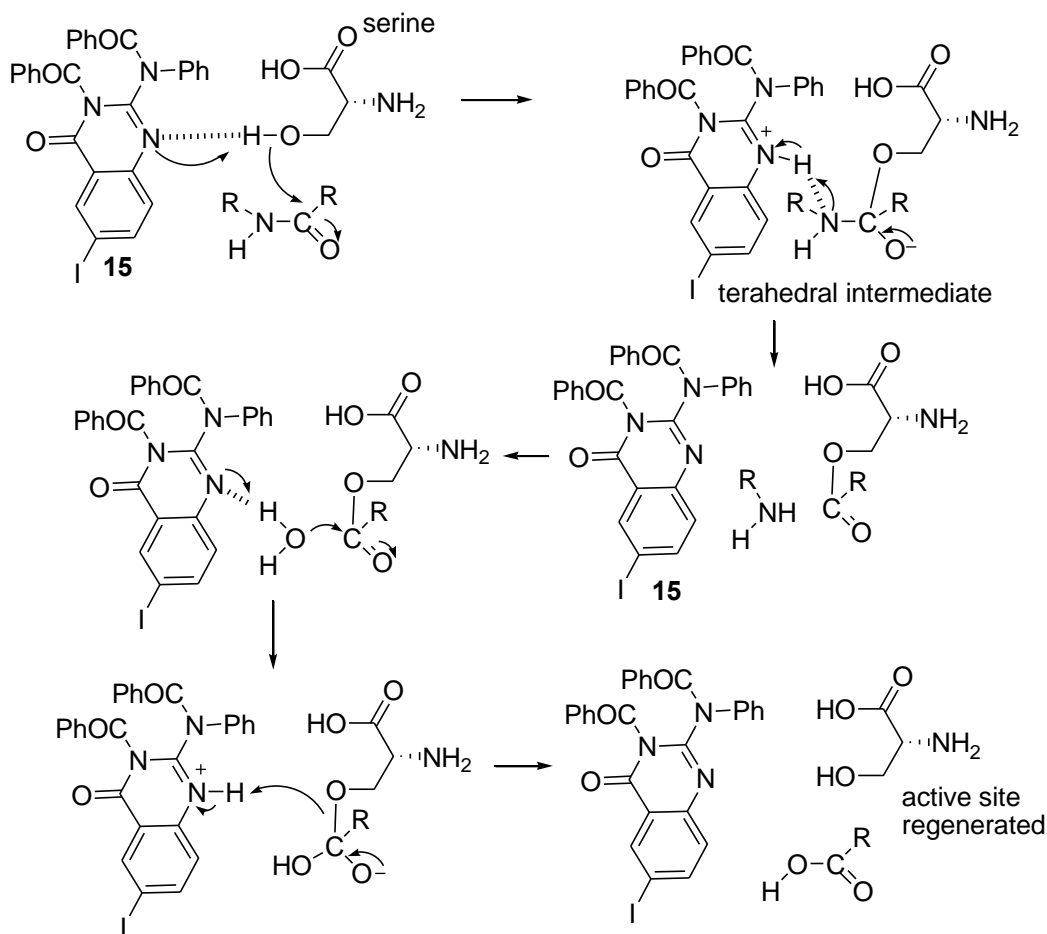


Figure 2

The quest for new antibiotic substances produced by microorganisms has stimulated investigations on comparatively simple synthetic compounds particularly those having structural features common to several of the natural antibiotics. Thus, oxazinylbenzohydrazide derivative (**6**) (similar to penicillin) exhibits low activity towards Gram positive bacteria (*Bacillus subtilis*) and Gram negative bacteria (*Escherichia coli*) and exhibits high activity towards yeast and fungi (*Candida albicans* and *Aspergillus fumigatus*). This is due to heteroatom ring opening by OH, NH₂ and SH groups present in bacteria or fungi and convert enzyme to inactive form.

The reactivity of the quinazolinone derivative (**14**) towards Gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), yeast and fungi (*Candida albicans* and *Aspergillus fumigatus*) is due to the formation of hydrogen bond between the organism and the tested compound. This is the same with *N*-(3-benzoylquinazoliny)-*N*-phenylbenzamide derivative (**15**) in which the reactivity increases due to the formation of strong hydrogen bond [C=O---HOE]. The high reactivity of compound **15** towards *Candida albicans* may be due to that a pair of electrons on the nitrogen atom in the ring which is similar to that of histidine has the ability to accept the hydrogen from the serine -OH group, thus coordinating the attack of the peptide bond. The carboxyl group on the acid e.g. aspartic acid in turn forms hydrogen bonds with compound **15**, making the nitrogen atom mentioned above much more electronegative. These results are depicted in Scheme 6.

p-Toylquinazolinone derivative (**10**) is similar to methqualine (exhibits high activity). While the activity of the dioxoisindoliny derivative (**24**), and (pentahydroxyhexylidene)acetohydrazide derivative (**23**) is due to carbamoyl moiety.



EXPERIMENTAL

All melting points were measured on a Gallenkamp melting point apparatus and were uncorrected. The infrared spectra were recorded using potassium bromide disks on a Pye Unicam SP-3-300 infrared spectrophotometer. ^1H NMR spectra were run at 300 MHz, on a Varian Mercury VX-300 NMR spectrometer, using TMS as an internal standard in deuterated dimethylsulphoxide. Chemical shifts δ are quoted in ppm. The mass spectra were recorded on Shimadzu GCMS-QP-1000EX mass spectrometers at 70 e.V. All the spectral measurements were carried out at the NMR laboratory of Cairo University, Egypt; the Micro analytical Center of Cairo University, Egypt; and the Main Defense Chemical Laboratory, Egypt. The elemental analyses were carried out at the Micro Analytical Center of Ain Shams University, Egypt. The antimicrobial activities were carried out at AL-Azhar University, Faculty of Agriculture; Egypt. All the chemical reactions were monitored by TLC. All the newly synthesized compounds gave satisfactory elemental analyses.

Starting Materials. 5-Iodoanthranilic acid (**1**)²⁰ was prepared by previously reported procedure. All other chemicals used in this study were commercially available.

5-Iodo-2-(3-phenylureido)benzoic acid (3). A mixture of 5-iodoanthranilic acid (**1**) (2.63 g, 0.01 mol) and phenyl isocyanate (**2**) (1.19 mL, 0.01 mol) was refluxed in dioxane (30 mL) for 3 h. The reaction mixture was poured onto ice and left overnight in a refrigerator, the solid that separated was filtered off, dried and recrystallized to give **3** (1.2 g, 31%); off white crystals; mp 170-171 °C (dioxane); IR (KBr) 3314, 3299, 1665, 1596, 1383 cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆) δ 6.47-8.22 (m, 8H), 9.81 (br s, 2H), 10.33 (br s, 1H); MS *m/z* 382 (M⁺, 64.20), 193 (100). Anal. Calcd for C₁₄H₁₁IN₂O₃: C, 44.00; H, 2.90; N, 7.33. Found: C, 44.20; H, 2.21; N, 7.03.

6-Iodo-2-(phenylamino)-4H-benzo[*d*][1,3]oxazin-4-one (4). A mixture of compound (**3**) (3.82 g, 0.01 mol) and acetic anhydride (2 mL) was heated on water bath for 1 h, the reaction mixture was left to cool, and the solid that separated was filtered off, dried to give compound **4** (2 g, 55%); yellow solid, mp 140-142 °C. (There are no data for this compound because it is a dynamic benzoxazinone).

***N*-(6-Iodo-4-oxo-2-(phenylamino)quinazolin-3(4*H*)-yl)benzamide (5).** **Method (A)** A mixture of benzoxazinone (**4**) (3.64 g, 0.01 mol) and benzoyl hydrazide (1.36 g, 0.01 mol) was refluxed for 3 h in EtOH (20 mL). The reaction mixture was concentrated to its half volume, cooled and the solid that separated was filtered off, dried and recrystallized to give **5**.

Method (B) The (oxazinyl)benzohydrazide derivative (**6**) was refluxed for 3 h in EtOH (20 mL). The reaction mixture was concentrated to its half volume, cooled and the solid that separated was filtered off, dried and recrystallized to give **5** (0.4 g, 8.2%); white crystals; mp 232-234 °C (EtOH); IR (KBr) 3201, 1668, 1633 cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆): δ 6.59-8.27 (m, 13H), 9.89 (br s, 1H), 10.49 (s, 1H); MS *m/z* 482 (M⁺, 9.71), 105 (100). Anal. Calcd for C₂₁H₁₅IN₄O₂: C, 52.30; H, 3.13; N, 11.62. Found: C, 51.90; H, 3.21; N, 11.57.

***N'*-(6-Iodo-4-oxo-2-(phenylamino)-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-2-yl)benzohydrazide (6).** A mixture of benzoxazinone (**4**) (3.64 g, 0.01 mol) and benzoyl hydrazide (1.36 g, 0.01 mol) in EtOH (20 mL) was stirred at room temperature for 24 h. The reaction mixture was concentrated to its half volume; and the solid that separated was filtered off, dried and recrystallized to give **6** (1.2 g, 24%); white crystals; mp 250-252 °C (EtOH); IR (KBr) 3235, 1753, 1701 cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆) δ 4.31 (br s, 2H), 6.95-8.14 (m, 13H), 11.80 (br s, 2H); MS *m/z* 500 (M⁺, 1.71), 105 (100). Anal. Calcd for C₂₁H₁₇IN₄O₃: C, 50.42; H, 3.43; N, 11.20. Found: C, 50.65; H, 3.21; N, 11.16.

Typical Procedure for the Preparation of Products 7 and 8.

A mixture of benzoxazinone (**4**) (3.64 g, 0.01 mol) and sodium azide (0.65 g, 0.01 mol) in DMF (30 mL) was refluxed for 3 h. The reaction mixture was cooled and poured onto ice. The solid that separated was filtered off, dried and separated by fractional crystallization from petroleum ether and EtOH to give compounds **7** and **8** respectively.

5-Iodo-2-oxo-N-phenyl-2,3-dihydro-1H-benzo[d]imidazole-1-carboxamide (7). (0.42 g, 11%); brown crystals; mp 220-222 °C (EtOH); IR (KBr) 3238, 1719, 1667 cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6) δ 7.29-8.27 (m, 8H), 11.63 (br s, 2H); MS m/z 378.98 (M^{\ddagger} , 7.40); 63 (100). Anal. Calcd for $\text{C}_{14}\text{H}_{10}\text{IN}_3\text{O}_2$: C, 44.35; H, 2.66; N, 11.08. Found: C 44.50; H, 2.46, N, 10.99.

5-Iodo-2-(5-(phenylamino)-1H-tetrazol-1-yl)benzoic acid (8) (0.3 g, 7.3%); white crystals; mp 202-204 °C (petroleum ether 60-80 °C); IR (KBr) 3235, 1689, 1651, 1089 cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6) δ 4.31 (br s, 1H), 7.04-8.16 (m, 8H), 11.63 (br s, 1H); MS m/z 406.99 (M^{\ddagger} , 7.19); 363 (100). Anal. Calcd for $\text{C}_{14}\text{H}_{10}\text{IN}_5\text{O}_2$: C, 41.30; H, 2.48; N, 17.20. Found: C, 41.27; H, 2.50; N, 17.15.

3-Amino-6-iodo-2-(phenylamino)quinazolin-4(3H)-one (9). A mixture of benzoxazinone (**4**) (3.64 g, 0.01 mol) and hydrazine hydrate (1 mL, 0.02 mol) in EtOH (30 mL) was heated under reflux for 3 h. The reaction mixture was concentrated and the solid that separated was filtered off, dried and crystallized to give **9** (1.2 g, 32%); white crystals, mp 110-112 °C (toluene/EtOH); IR (KBr) 3293, 3259, 3194, 1663, 1600 cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6) δ 2.51 (br s, 2H), 6.98-7.58 (m, 8H), 9.85 (br s, 1H); MS m/z 378 (M^{\ddagger} , 0.00), 363 ($\text{M}^+-\text{NH}_2+\text{H}$) (7.73), 80 (100). Anal. Calcd for $\text{C}_{14}\text{H}_{11}\text{IN}_4\text{O}$: C, 44.46; H, 2.93; N, 14.82. Found: C, 44.50; H, 2.71; N, 14.70.

6-Iodo-2-(phenylamino)-3-p-tolylquinazolin-4(3H)-one (10). A mixture of benzoxazinone (**4**) (3.64 g, 0.01 mol) and *p*-toluidine (1.07 g, 0.01 mol) was refluxed for 3 h in EtOH (30 mL), the solid that separated after concentration was filtered off, dried, and recrystallized to give **10** (1.2 g, 26%); brown crystals; mp 127-129 °C (MeOH); IR (KBr) 3193, 1666, 1602 cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6) δ 2.24 (s, 3H), 6.99-8.27 (m, 12H), 9.68 (br s, 1H); MS m/z 453.03 (M^{\ddagger} , 0.00), 376 ($\text{M}^+-\text{C}_6\text{H}_5$) (8.44), 245 (100). Anal. Calcd for $\text{C}_{21}\text{H}_{16}\text{IN}_3\text{O}$: C, 55.64; H, 3.56; N, 9.27. Found: C, 55.30; H, 3.29; N, 9.14.

3-Hydroxy-6-iodo-2-(phenylamino)quinazolin-4(3H)-one (11). A mixture of benzoxazinone (**4**) (3.64 g, 0.01 mol) and hydroxylamine hydrochloride (0.69 g, 0.01 mol) was refluxed for 3 h in pyridine (20 mL). The reaction mixture was cooled and then poured onto ice/HCl. The solid that separated was filtered off, dried and then recrystallized to give **11** (1.53 g, 40%); off white crystals; mp 240-242 °C (toluene/EtOH); IR (KBr) 3438, 3237, 1686, 1653 cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6) δ 7.29-8.27 (m, 8H), 10.91 (br s, 1H), 11.60 (br s, 1H); MS m/z 378.98 (M^{\ddagger} , 0.00), 364 (100), 363 (14.28). Anal. Calcd for $\text{C}_{14}\text{H}_{10}\text{IN}_3\text{O}_2$: C, 44.35; H, 2.66; N, 11.08. Found: C, 44.0; H, 2.29; N, 11.04.

6-Iodo-4-oxo-2-(phenylamino)quinazolin-3(4H)-yl acetate (12). A mixture of quinazolinone (**11**) (3.79 g, 0.01 mol) and acetyl chloride (20 mL) was heated for 1 h, the reaction mixture was left to cool, poured onto ice, then filtered off, dried and recrystallized to give **12** (0.5 g, 12%); off white crystals; mp 230-232 °C (EtOH); IR (KBr) 3360, 1734, 1715, 1685, 1228 cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6) δ 2.12

(s, 3H), 7.85-8.27 (m, 8H), 10.88 (br s, 1H); MS m/z 420.99 (M^+ , 0.00), 365 (0.48), 305 (40.24), 263 (100). Anal. Calcd for $C_{16}H_{12}IN_3O_3$: C, 45.63; H, 2.87; N, 9.98. Found: C, 45.57; H, 2.69; N, 9.84.

Ethyl 2-(6-iodo-4-oxo-2-(phenylamino)quinazolin-3(4H)-yloxy)acetate (13). A solution of quinazolinone (**11**) (3.78 g, 0.01 mol) in dry acetone (30 mL) was refluxed with ethyl chloroacetate (3.32 g, 0.03 mol), and potassium carbonate (4.14 g, 0.03 mol) for 24 h on water bath. The solvent was evaporated, and the reaction mixture was. The solid that separated out was filtered off, dried and then recrystallized to give **13** (1.2 g, 26%); yellow crystals; mp 90-92 °C (petroleum ether 60-80 °C); IR (KBr) 3338, 1742, 1709, 1685; 1H NMR (300 MHz, DMSO- d_6) δ 1.32 (t, $J = 6.0$ Hz, 3H), 4.18 (q, $J = 6.0$ Hz, 2H), 4.92 (s, 2H), 7.43-8.18 (m, 7H), 8.19 (s, 1H), 10.28 (br s, 1H); MS m/z 465 (M^+ , 3.27), 77 (100). Anal. Calcd for $C_{18}H_{16}IN_3O_4$: C, 46.47; H, 3.47; N, 9.03. Found: C, 46.48; H, 3.65; N, 9.05.

6-Iodo-2-(phenylamino)quinazolin-4(3H)-one (14). A solution of benzoxazinone (**4**) (3.64 g, 0.01 mol) in formamide (20 mL) was heated under reflux for 2 h. The reaction mixture was diluted with cold water and the solid that separated out was filtered off, dried and recrystallized to give **14** (2 g, 55%); green needles crystals; mp 270-272 °C (toluene); IR (KBr) 3314, 3164, 1683, 1613 cm^{-1} ; 1H NMR (300 MHz, DMSO- d_6): δ 2.33 (br s, 1H), 7.34-8.38 (m, 7H), 8.39 (s, 1H), 12.38 (s, 1H); MS m/z 363 (M^+ , 62.83), 69 (100.00). Anal. Calcd for $C_{14}H_{10}IN_3O$: C, 46.30; H, 2.78; N, 11.57. Found: C, 46.00; H, 2.51; N, 11.20.

***N*-(3-Benzoyl-6-iodo-4-oxo-3,4-dihydroquinazolin-2-yl)-*N*-phenylbenzamide (15).** A mixture of quinazolinone (**14**) (3.63 g, 0.01 mol) and benzoyl chloride (17.5 mL, 0.15 mol) was refluxed for 3 h in pyridine (30 mL). The reaction mixture was cooled and then poured onto ice /HCl, the solid that separated was filtered off, dried and recrystallized to give **15** (1.6 g, 28%); yellow crystals; mp 260-262 °C (EtOH/dioxane); IR (KBr) 1693, 1616 cm^{-1} ; 1H NMR (300 MHz, DMSO- d_6): δ 7.47-7.96 (m, 18H); MS m/z 571.03 (M^+ , 0.00), 494 (22.07), 105 (100). Anal. Calcd for $C_{28}H_{18}IN_3O_3$: C, 58.86; H, 3.18; N, 7.35; Found: C, 58.97; H, 3.15; N, 7.24.

3-Acetyl-6-iodo-2-(phenylamino)quinazolin-4(3H)-one (16). A mixture of quinazolinone (**14**) (3.63 g, 0.01 mol) and acetyl chloride (10 mL) was refluxed for 1 h, cooled, poured onto ice/H₂O, stirred for 1 h and then the solid that separated was filtered off, dried and recrystallized to give **16** (2.3 g, 63.6%); yellow crystals; mp > 300 °C (toluene); IR (KBr) 3317, 1742, 1680, 1619 cm^{-1} ; 1H NMR (300 MHz, DMSO- d_6): δ 2.49 (s, 3H), 7.35-8.33 (m, 8H), 12.30 (br s, 1H); MS m/z 405 (M^+ , 69.09), 344 (100). Anal. Calcd for $C_{16}H_{12}IN_3O_2$: C, 47.43; H, 2.99; N, 10.37. Found: C, 47.30; H, 2.71; N, 10.63.

Ethyl 2-(6-iodo-4-oxo-2-(phenylamino)quinazolin-3(4H)-yl)acetate (17). A solution of quinazolinone (**14**) (3.63 g, 0.01 mol) in dry acetone (30 mL) was refluxed with ethyl chloroacetate (3.32 g, 0.03 mol),

and potassium carbonate (4.14 g, 0.03 mol) for 24 h on water bath. The solvent was evaporated, and the reaction mixture was poured on water. The solid that separated out was filtered off, dried and then recrystallized to give **17** (3.36 g, 75%); yellow crystals, mp 98-100 °C (EtOH/dioxane); IR (KBr) 3338, 1735, 1674 cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆): δ 2.33 (t, *J* = 6.0 Hz, 3H), 4.56 (q, *J* = 6.0 Hz, 2H), 4.82 (s, 2H), 7.28-8.41 (m, 8H), 12.25 (br s, 1H); MS *m/z* 449 (M⁺, 73.68), 393 (100). Anal. Calcd for C₁₈H₁₆N₃O₃: C, 48.12; H, 3.59; N, 9.35. Found: C, 48.0; H, 3.31; N, 9.13.

2-(6-Iodo-4-oxo-2-(phenylamino)quinazolin-3(4H)-yl)acetohydrazide (18). A mixture of compound (**17**) (4.49 g, 0.01 mol) and hydrazine hydrate (0.50 mL, 0.01 mol) in EtOH (30 mL) was heated under reflux for 3 h, after cooling the obtained solid was filtered off, dried and recrystallized to give **18** (2.7 g, 62%); yellow crystals; mp 263-265 °C (butanol); IR (KBr) 3352, 3308, 3282, 1667, 1606, cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆): δ 4.30 (s, 2H), 4.60 (s, 2H), 7.47-8.40 (m, 8H), 9.41 (br s, 2H); MS *m/z* 435 (M⁺, 59.46), 386 (100). Anal. Calcd for C₁₆H₁₄N₅O₂: C, 44.16; H, 3.24; N, 16.09. Found: C, 44.00; H, 3.21; N, 16.03.

6-Iodo-3-(2-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-2(phenylamino)quinazolin-4(3H)-one (19). A mixture of acetohydrazide (**18**) (4.35 g, 0.01 mol) and ethyl acetoacetate (1.3 mL, 0.01 mol) was refluxed for 3 h in butanol (20 mL). The solid that separated out after concentration was filtered off, dried and recrystallized to give **19** (0.99 g, 20%); off white crystals, mp > 300 °C (EtOH/dioxane); IR (KBr) 3196, 1680, 1628, 1604 cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆): δ 2.08 (s, 3H), 4.82 (s, 2H), 5.21 (s, 2H), 7.38-8.40 (m, 8H), 10.50 (d, *J* = 15 Hz, 1H, the rate of exchange of NH is slow and the proton couples with the nitrogen atom); MS *m/z* 501 (M⁺, 41.57), 98 (100). Anal. Calcd for C₂₀H₁₆N₅O₃: C, 47.92; H, 3.22; N, 13.97. Found: C, 47.82; H, 3.02; N, 13.77.

3-(2-(3,5-Dimethyl-1H-pyrazol-1-yl)-2-oxoethyl)-6-iodo-2-(phenylamino)quinazolin-4(3H)-one (20). A mixture of compound (**18**) (4.35 g, 0.01 mol) and acetylacetone (1.0 mL, 0.01 mol) was refluxed for 3 h in butanol (20 mL). The solid that separated after concentration was filtered off, dried, and recrystallized to give **20** (0.7 g, 14%); off white crystals; mp > 300 °C (dioxane); IR (KBr) 3193, 1680, 1630, 1605 cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆): δ 3.56 (s, 3H), 3.64 (s, 3H), 4.74 (s, 2H), 4.84 (s, 1H), 7.30-8.39 (m, 8H), 10.53 (d, 1H, the rate of exchange of NH is slow and the proton couples with the nitrogen atom); MS *m/z* 499.05 (M⁺, 0.00), M⁺² (61.36), 96 (100). Anal. Calcd for C₂₁H₁₈N₅O₂: C, 50.52; H, 3.63; N, 14.03. Found: C, 50.22; H, 3.33; N, 14.10.

N'-Benzylidene-2-(6-iodo-4-oxo-2-(phenylamino)quinazolin-3(4H)-yl)acetohydrazide (21). A mixture of compound (**18**) (4.35 g, 0.01 mol) and benzaldehyde (1.06 mL, 0.01 mol) was refluxed for 3 h in butanol (20 mL). The solid that separated after concentration was filtered off, dried, and recrystallized to give **21** (1.2 g, 23%); brown crystals; mp 250-252 °C (toluene/EtOH); IR (KBr) 3213, 1703, 1661, 1608

cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆): δ 4.79 (s, 2H), 5.23 (s, 2H), 5.31 (s, 1H), 5.75 (br s, 1H), 7.14-8.42 (m, 14H), 11.82 (d, 1H, the rate of exchange of NH is slow and the proton couples with the nitrogen atom); MS *m/z* 523 (M⁺, 33.33), 75 (100). Anal. Calcd for C₂₃H₁₈IN₅O₂: C, 52.79; H, 3.47; N, 13.38. Found: C, 52.52; H, 3.27; N, 13.10.

2-(6-Iodo-4-oxo-2-(phenylamino)quinazolin-3(4*H*)-yl)-*N*-(4-oxo-2-phenylthiazolidin-3-yl)acetamide (22). A mixture of compound (21) (5.23 g, 0.01 mol) and thioglycolic acid (0.70 mL, 0.01 mol) in benzene (20 mL) was refluxed for 2 h. The reaction mixture was cooled and concentrated to its half volume; the solid that separated out was filtered off, dried and recrystallized to give **22** (0.4 g, 6.7%); yellow crystals; mp 148-150 °C (EtOH); IR (KBr) 3234, 1687, 1651 cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆): δ 3.72 (br s, 1H), 4.91 (s, 2H), 5.23 (s, 1H), 5.31 (s, 1H), 7.29-8.70 (m, 13H), 8.42 (br s, 1H), 11.83 (br s, 1H); MS *m/z* 597.03 (M⁺, 0.00), 535 (2.14), 258 (100). Anal. Calcd for C₂₅H₂₀IN₅O₃S: C, 50.26; H, 3.37; N, 11.72, S, 5.37. Found: C, 50.02; H, 3.30; N, 11.70; S, 5.21.

2-(6-Iodo-4-oxo-2-(phenylamino)quinazolin-3(4*H*)-yl)-*N'*-(2,3,4,5,6-pentahydroxyhexylidene)acetylhydrazide (23). A mixture of compound (18) (4.35 g, 0.01 mol), and glucose (1.80 g, 0.01 mol) in butanol (30 mL) was refluxed for 3 h. The reaction mixture was cooled and then concentrated to its half volume; the solid that separated out was filtered off, dried and recrystallized to give **23** (0.6 g, 10%); white crystals; mp 181-83 °C (MeOH); IR (KBr) basin peak centered at 3344, 1732, 1684 cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆): δ 3.13-3.30 (m, 3H), 3.41-3.44 (m, 2H), 3.90 (br s, 1H), 4.16 (d, 1H), 4.38 (br s, 3H), 4.91 (s, 2H), 5.72 (br s, 1H), 7.38-7.43 (m, 4H), 7.49 (d, 1H), 8.06-8.34 (m, 3H), 8.40 (s, 1H), 9.04 (br s, 1H), 9.86 (br s, 1H); MS *m/z* 597.07 (M⁺, 0.00), 80 (100.00). Anal. Calcd for C₂₂H₂₄IN₅O₇: C, 44.23; H, 4.05; N, 11.72. Found: C, 43.19; H, 3.87; N, 12.50.

***N*-(1,3-Dioxoisindolin-2-yl)-2-(6-iodo-4-oxo-2-(phenylamino)quinazolin-3(4*H*)-yl)acetamide (24).** A mixture of compound (18) (4.35 g, 0.01 mol) and phthalic anhydride (1.48 g, 0.01 mol) in butanol (30 mL) was refluxed for 3 h. The reaction mixture was cooled and concentrated to its half volume; the solid that separated out was filtered off, dried and recrystallized to give **24** (0.4 g, 7%); brown crystals; mp 287-289 °C (EtOH); IR (KBr) 3171, 1663 cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆): δ 4.60 (s, 2H), 7.08-8.43 (m, 12H), 9.40 (br s, 1H) 10.50 (br s, 1H); MS *m/z* 565.02 (M⁺, 0.00), 421 (5.10), 64 (100). Anal. Calcd for C₂₄H₁₆IN₅O₄: C, 50.99; H, 2.85; N, 12.39. Found: C, 50.80; H, 2.65; N, 12.30.

Biological activities

Antimicrobial activity

The standardized disc-agar diffusion method^{21,22} was followed to determine the activity of the synthesized compounds against the tested microorganisms.

Test Organisms

Cultures of the following microorganism were used in the test:

Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6635),

Gram-negative bacteria: *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028),

Yeast: *Candida albicans* (ATCC 10231) and Fungus: *Aspergillus fumigatus*.

Screening for the antimicrobial potential:

Preparation of the tested compounds

The tested compounds were dissolved in dimethylformamide (DMF) solvent and prepared in two concentrations; 100 and 50 mg/mL and then 10 μ L of each preparation was dropped on disk of 6 mm in diameter and the concentrations became 1 and 0.5 mg/disk respectively. In the case of insoluble compounds, the compounds were suspended in DMF and vortexed then processed.

Testing for antibacterial and yeasts activity:

Bacterial cultures were grown in nutrient broth medium at 30 °C. After 16 h of growth, each microorganism, at a concentration of 10^8 cells/mL, was inoculated on the surface of Mueller-Hinton agar plates using sterile cotton swab. Subsequently, uniform size filter paper disks (6 mm in diameter) were impregnated by equal volume (10 μ L) from the specific concentration of dissolved compounds and carefully placed on the surface of each inoculated plate. The plates were incubated in the upright position at 36 °C for 24 h. Three replicates were carried out for each extract against each of the test organism. Simultaneously, addition of the respective solvent instead of dissolved compounds was carried out as negative controls. After incubation, the diameters of the growth inhibition zones formed around the disc were measured with transparent ruler in millimeter, averaged and the mean values were tabulated.

Testing for anti-fungal activity:

Active inoculum for experiments were prepared by transferring many loopfuls of spores from the stock cultures to test tubes of sterile distilled water (SDW) that were agitated and diluted with sterile distilled water to achieve optical density corresponding to 2.0×10^5 spore/mL inoculum of 0.1% suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min then the same procedure was followed as described above.

Standard references:

The antibiotics chloramphenicol, Cephalothin, cycloheximide were used as standard references in the case of Gram negative bacteria, Gram positive bacteria, yeasts and fungi respectively.

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