BIOLOGICAL ACTIVITY OF SOME NEWLY SYNTHESIZED HYDRAZONE DERIVATIVES DERIVED FROM (DICYCLOPROPYLMETHYLENE)HYDRAZONE

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A series of new hydrazone derivatives were synthesized using dicyclopropyl ketone as starting material. Unexpected reaction of salicylaldehyde with (dicyclopropylmethylene)hydrazine yielded the appropriate bis(salicylaldehyde azine). Treatment of the (dicyclopropylmethylene)hydrazine with the appropriate coupling agents gave the corresponding imides, imidazolones, oxazolones, quinazolinones and triazoles, respectively. The synthesized compounds were characterized using IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data. These newly formed products were tested for their antibacterial and antifungal activities and most of the compounds showed high activity compared with Ciprofloxacin as positive controls. In addition, the compounds were also, examined for their anticancer activities against breast cancer cell line (MCF7) compared with cisplatin as a positive control. The novel synthesized compounds showed satisfactory activity against (MCF7) with mean IC_{50} values ranging from 21.5 to 100 μM.

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Introduction

Hydrazones have diverse interesting biological activities[1] including antitumor,[2–4] anti-inflammatory,[5] antimalarial,[6] antimicrobial,[7,8] insecticidal,[9] antiplatelet,[10] antibacterial, antifungal, antimycobacterial, cytotoxic and cytostatic activities.[11,12] Cu(II) phenanthroline hydrazone complexes are able to cleave DNA and show cytotoxic activity against cancer cells.[13] Aminoguanidine hydrazones (AGH's) have potential important towards developing focused adjuvants for antibiotic drug therapies against bacterial multidrug resistance.[14] These compounds also evaluated for their in vitro α-glucosidase inhibitory activity.[15] Hydrazones have been used to design materials that are used for pH-responsive drug delivery systems and these hydrazones were characterized by different spectral methods. Therefore there is scope to utilize these dendrimers to form hydrazone conjugates with the drugs (anticancer as well as antimicrobial) for more effective and targeted delivery in acidic environments.[16] Hydrazones have an attractive therapeutic approach, which is widely applied in the treatment of many diseases. For example, Alzheimer's disease (AD).[17] Moreover, the significance of hydrazones is due to their ability to extensively used as intermediates for the synthesis of heterocyclic compounds of pharmaceutical interests[18,19] and as transition metal chelators.[20,21] Therefore, and in view of these findings we report here in the utilization of (dicyclopropylmethylene)hydrazone (2) which has previously been prepared by the reaction of dicyclopropyl ketone and hydrazine hydrate [22] as a primitive compound for synthesis of novel of a series of new heterocyclic compounds with a biologically active pharmacophore (=NH–N-CH–) followed by screened for their antibacterial, antifungal and anticancer activities against breast cancer cell line (MCF7).

Experimental

All compounds were used without any purification or treatment. Reagents were used synthesized according to published literature. Thin-layer chromatography (TLC) was used to monitor the reaction and emphasizes the purity of the products. Melting points are recorded in Gallenkamp electric melting point apparatus and are uncorrected. The IR spectra $\acute{\upsilon}$ (cm^{-1}) were measured on FT-IR spectrophotometer in potassium bromide (KBr). The ^{1}H-NMR and ^{13}C-NMR spectra were measured were measured in DMSO-d6 as solvent at 500 MHz on a JNM-ECA500II NMR spectrometer using tetramethylsilane (TMS) as an internal reference and chemical shifts are expressed as δ ppm. The mass spectra (EI) were recorded on 70 eV with Kratos MS equipment.

Synthesis of (dicyclopropylmethylene)hydrazone (2)

Hydrazine hydrate (1 mmol) was added to an equimolar amount of a solution of dicyclopropyl ketone (1 mmol) in 15 mL of absolute ethanol. The reaction mixture was heated under reflux for 5 h, evaporate the excess ethanol and then left to cool down to 0°c. The solid product was obtained by pouring onto ice-water with stirring, filtered off, dried, the product is sufficiently pure and it didn't need purification to give 2 as Colorless powder; yield 92 %; m.p. 85° C. IR (KBr, ν/cm^{-1}): 3425, 3395 (NH_2), 1604 (C=N).^{1}H NMR (DMSO-d6) δ ppm 0.6-1.07 (m, 8H, $4CH_2$), 2.25 (m, 4H, $2CH,NH_2$). ^{13}C-NMR (DMSO-d6): δ ppm 5.6, 7.24, 9.36, 11.45 and 165.8. MS (EI): (m/z, %), 124 (M^{+}-1, 1), 108 (100).

General procedure for the synthesis of 3-10

A mixture of equimolar amount of **2** (1 mmol) and appropriate aldehyde (p-methoxybenzaldehyde, p-nitrobenzaldehyde, furfural, isatin or salicylaldehyde) (1

mmol) in absolute ethanol (30 ml) was refluxed in water bath for 6-8 h. the reaction mixture was left to cool, the resulting precipitate was filtered off and recrystallized from ethanol to give **3-8**.

1-(Dicyclopropylmethylene)-2-(4-methoxybenzylidene)-hydrazine (3a)

Yellow needles; yield 78 %; mp 164-6 °C IR (KBr, ν/cm^{-1}): 2967-2840 (CH aliph.), 1659, 1600 (2C=N).^{1}H NMR (DMSO-d6) δ ppm 0.1-1.06 (m, 10H, H-aliph), 3.79 (s, 3H, OCH_3) and 7.035-7.8 (m, 4H, H-Ar) 8.6 (s, 1H, CH=N).^{13}C-NMR (DMSO-d6): δ ppm 5.6, 7.24, 9.36, 11.45 and 165.8. MS (EI): (m/z, %), 241 (M^+-1, 9.08), 160 (100).

1-(Dicyclopropylmethylene)-2-(4-nitrobenzylidene)hydrazine (3b)

Yellow crystals; yield 92 %; mp. >250 °C. IR (KBr, ν/cm^{-1}): 2961-2830 (CH aliph.), 1609 (2C=N). ^{1}H NMR (DMSO-d6) δ ppm 0.12-1.4 (m, 10H, H-aliph), 6.8-7.1 (m, 4H, H-Ar) and 8.3 (s, 1H, CH=N). MS (EI): (m/z, %), 257 (M^+, 23).

1-(Dicyclopropylmethylene)-2-(furan-2-ylmethylene)hydrazine (3c)

Bale yellow needles; yield 81 %; mp 104 °C, IR (KBr, ν/cm^{-1}): 2927-2850 (CH aliph.), 1656-1651 (2C=N).^{1}H NMR (DMSO-d6) δ ppm 0.1-1.6 (m, 10H, H-aliph), 6.5-7.5 (m, 3H, H-Ar) and 8.1 (s, 1H, CH=N). MS (EI): (m/z, %), 202 (M^+, 60)

3-((Dicyclopropylmethylene)hydrazono)indolin-2-one (4)

Dark red crystals; yield 88 %; mp >300 °C. the reaction mixture with few drops of acetic acid as catalyst. IR (KBr, ν/cm^{-1}):3277(NH), 1723, 1613 (C=O, C=N).^{1}H NMR (DMSO-d6) δ ppm 0.9-1.3 (m, 10H, H-aliph), 6.8-7.5 (m, 4H, H-Ar) and 10.9(s, 1H, NH). ^{13}C-NMR (DMSO-d6): δ ppm 7.36, 9.2, 13.13, 110.6, 111.12, 122.05, 127.8, 128.2, 134.4, 144.73, 145.19 and 163.4 MS (EI): (m/z, %), 253 (M^+, 100).

Ethyl-3-((dicyclopropylmethylene)hydrazono)butanoate (5)

Yellow crystals; yield 68 %; mp190 °C. IR (KBr, ν/cm^{-1}):1683, 1613 (C=O, C=N).^{1}H NMR (DMSO-d6) δ ppm 0.9-1.0 (m, 10H, H-aliph), 1.1(t, 3H, CH_3), 2.2(S, 3H, CH_3), 1.1(t, 3H, CH_3), 2.2(S, 2H, CH_2)and 4.0(q, 2H, CH_2 ester). ^{13}C-NMR (DMSO-d6): δ ppm 12.8, 14.3, 17.7, 58.7, 103.8, 11.9, 138.6, 149.8, 159.7, and 166.8 MS (EI): (m/z, %), 236 (M^+, 2.5).

2,2'-(Hydrazine-1,2-diylidenebis(methanylylidene))-diphenol(10)

Bright yellow crystals ; yield 95 %; mp 205-7 °C

IR (KBr, ν/cm^{-1}):3445(OH), 1622 (2 C=N).^{1}H NMR (DMSO-d6) δ ppm 6.9-7.6(m, 8H, H-Ar), 8.9(s, 2H, CH=N) and 11.1(s, 2H, OH). MS (EI): (m/z, %), 240 (M^+, 17) and 185(100).

General procedure for the synthesis of 7a, 8a

Thiosemicarbazide (1 mmol) was added to an equimolar amounts of solution of **3a** (1 mmol) in 25 mL of absolute ethanol and few drops of acetic acid. The reaction mixture was heated under reflux for 12 h then the precipitated crude product was filtered off.

4-((Dicyclopropylmethylene)amino)-5-(4-methoxyphenyl)-4,5-dihydro-1H-1,2,4-triazol-3-amine(7a)

White crystals (from ethanol) 62 %, m.p 160-162 °C. IR (KBr, ν/cm^{-1}): 1793,1725, 1653 (C=O,2 C=N).^{1}H NMR (DMSO-d6) δ ppm 1.02 1.4 (m, 10H, H-aliph) and 3.8(s, 3H, OCH_3), 5.8 (s, 1H, CH), 6.7, 7.8 (m, 4H, H-Ar), 8.6 (s, 2H, NH_2) and 9.8 (s, 1H, NH). MS (EI): (m/z, %), 268 (M^+-OCH_3, 6.6).

5'-Amino-4'-((dicyclopropylmethylene)amino)-2',4'-dihydro-spiro[indoline-3,3'-[1,2,4]triazol]-2-one (8a)

Yellow crystals (from ethanol) 75 %, m.p 238-240 °C IR (KBr, ν/cm^{-1}): 3419, 3351(2NH), 3259, 3190(NH_2), 1679, 1613 (C=O, C=N).^{1}H NMR (DMSO-d6) δ ppm 1.0-1.055 (m, 10H, H-aliph), 6.8-7.3 (m, 4H, H-Ar), 10.5 (s, 1H, NH), 10.6 (s, 1H, NH) and 11.1 (s, 2h, NH_2) .^{13}C-NMR (DMSO-d6): δ ppm 10-20, 109.9, 111.03, 117.4, 119.98,162.7, 178.66 and 181.13 MS (EI): (m/z, %), 313 (M^++2, 8.4).

General procedure for the synthesis of 7b, 8b

A mixture of **4** (1 mmol) and of benzohydrazide (1 mmol) in 20 ml absolute ethanol in the presence of alcoholic potassium hydroxide (1.5 mmol) was refluxed for 22 h. The reaction mixture was cooled, then poured into ice-water, then it was acidified with diluted HCl to pH 6. The formed crude product was filtered off,

1,1-Dicyclopropyl-N-(5-(4-methoxyphenyl)-3-phenyl-1,5-dihydro-4H-1,2,4-triazol-4-yl)methanimine (7b)

Brown crystals (from ethanol) 62 %, m.p >250 °C IR (KBr, ν/cm^{-1}): 3419, 3351(2NH), 3259, 3190(NH_2), 1679, 1613 (C=O, C=N).^{1}H NMR (DMSO-d6) δ ppm 1.0-1.055 (m, 10H, H-aliph), 6.8-7.3 (m, 4H, H-Ar), 10.5 (s, 1H, NH), 10.6 (s, 1H, NH) and 11.1 (s, 2h, NH_2) .^{13}C-NMR (DMSO-d6): δ ppm 10-20, 109.9, 111.03, 117.4, 119.98,162.7, 178.66 and 181.13 MS (EI): (m/z, %), 313 (M^++2, 8.4)

4'-((Dicyclopropylmethylene)amino)-5'-phenyl-2',4'-dihydro-spiro[indoline-3,3'-[1,2,4]triazol]-2-one (8b)

Yellow crystals (from ethanol) 62 %, m.p 262-264 °C. IR (KBr, ν/cm^{-1}): 3349 (NH), 1685, 1601 (2C=N).^{1}H NMR (DMSO-d6) δ ppm 0.9-2.1 (m, 10H, H-aliph), 3.8 (s, 3H, CH_3) and 7-7.9 (m, 9H, H-Ar) MS (EI): (m/z, %) 360 (M^+- 1, 15.1).

Synthesis 2-(3-amino-4-((2-hydroxybenzylidene)amino)-4,5-dihydro-1H-1,2,4- triazol-5-yl)phenol (11a)

Thiosemicarbazide (1 mmol) was added to equimolar amounts of the solution of **10** (1 mmol) in 25 mL of absolute

ethanol and few drops of acetic acid. The reaction mixture was heated under reflux for16 h, the precipitated of the crude product was filtered and further purified by recrystallization from methanol to give **11a** as faint yellow crystals; yield 95 %; mp 226-8 ºC. IR (KBr, ν/cm^{-1}):3443, 3320(NH_2),3173, 3137 (2CH=N), 1723, 1613 (C=N).^{1}H NMR (DMSO-d6) δ ppm 6.7-8.0 (m, 8H, H-Ar), 8.3 (s, 1H, CH=N),9.8(s, 1H, NH_2)and 11.3(s, 1H, OH). ^{13}C-NMR (DMSO-d6): δ ppm 116, 119.34, 120.37, 126.7, 131.17, 139.7, 156.4 and 177.6 MS (EI): (m/z, %), 297 (M^+, 2.5), 195(100).

Synthesis of 2-(4-((2-hydroxybenzylidene)amino)-3-phenyl-4,5-dihydro-1H-1,2,4-triazol-5-yl)phenol(11b)

A mixture of **10** (1mmol) and (1 mmol) of benzohydrazide in 20 ml absolute ethanol in the presence of alcoholic potassium hydroxide (1.5 mmol) was refluxed for 8 h. The reaction mixture was cooled, then poured into ice-water, then it was acidified with dilute HCl to pH 6. The formed crude product was filtered off, dried, and crystallized from methanol to give (55 %) of **11b** as yellow crystals; yield 54 %; mp. 198-200 ºC.

IR (KBr, ν/cm^{-1}): 1689,1623 (2 C=N).^{1}H NMR (DMSO-d6) δ ppm 6.5-7.1(m, 8H, H-Ar), 8.5(s, H, CH=N) and 11.1(s, 2H, 2OH). MS (EI): (m/z, %), 356 (M^+, 20).

General procedure for the synthesis of 12, 13

A mixture of dicyclopropyl ketone **1** (1 mmol) and cyanoacetic acid hydrazide (1 mmol) in 25 ml absolute ethanol was refluxed for 3 h on a water bath. After leaving the mixture to cool to room temperature, the white precipitate that formed was filtered off to be the crude product of **12**. The filtrate was cooled to 0ºc the brown pellets that precipitated was filtered off to afford **13.**

2-Cyano-N'-(dicyclopropylmethylene)acetohydrazide (12)

White crystals (from ethanol) 65 %, m.p 125-126 ºC. IR (KBr, ν/cm^{-1}):3198 (NH), 2264 (cyano), 1679 (C=N).^{1}H NMR (DMSO-d6) δ ppm 0.6-2.03 (m, 10H, H-aliph), 3.9 (s, 2H, CH_2) and 10.86(s, 1H, NH). ^{13}C-NMR (DMSO-d6): δ ppm 5.92, 6.86, 10.5, 11.03, 24.57, 116.24, 157.92 and 165.03 MS (EI): (m/z, %), 191 (M^+, 13), 123(100).

5-Amino-1-(dicyclopropylmethyl)-1,2-dihydro-3H-pyrazol-3-one (13)

Brown pellets (from ethanol) 33 %, m.p 210 °C. IR (KBr, ν/cm^{-1}):3337(NH), 3166, 3100 (NH_2), 1689, 1654 (C=O, C=N).^{1}H NMR (DMSO-d6) δ ppm 0.8-1.8 (m, 10H, H-aliph), 4.1(s, 1H, CH-N), 5.6 (s, 1H, NH), 5.8 (S, 1H, H-Ar) and 9.8(s, 2H, NH_2). ^{13}C-NMR (DMSO-d6): δ ppm 18.6, 36.27, 56.08, 74.23, 157.0, 159.3 and 171.7. MS (EI): (m/z, %), 193 (M^+, 1.5), 69(100).

Synthesis of 1-(dicyclopropylmethylene)-3-(2-methyl-4-oxoquinazolin-3(4H)-yl)thiourea (15)

A mixture of equimolar amount of dicyclopropyl ketone (1 mmol) and thiourea derivative **14** (1 mmol) in absolute ethanol (25 ml) was refluxed in water bath for 12 hr. the reaction mixture was left to cool, the resulting precipitate was Filtered off and recrystallized from ethanol to give **15** as brown needles in 45 % yield, mp 135-2 °C. IR (KBr, ν/cm^{-1}):3432 (NH), 1675, 1660 (C=O, C=N).^{1}H NMR (DMSO-d6) δ ppm 1.1-1.8 (m, 13H, H-aliph, CH_3), 6.4-7.3 (m, 4H, H-Ar) and 8.2 (s, 1H, NH). MS (EI): (m/z, %), 326 (M^+, 100).

General procedure for the synthesis of 16-20

To a solution of **2** (5 mmol) and 25 ml of glacial acetic acid, (5 mmol) of phthalic anhydride, maleic anhydride, succinic anhydride, 4-benzylidene-2-methyloxazol-5(4H)-one, 4-benzylidene-2-phenyloxazol-5(4H)-one or 2-methylbenzoxazinone was added. The reaction mixture was refluxed for 6-24 h. The solution was poured on ice-cold water. The formed precipitate was filtered, washed with water, dried and crystallized from the appropriate solvent.

2-(2-(Dicyclopropylmethylene)hydrazine-1-carbonyl)benzoic acid (16)

White crystals (from acetic acid) 72 %, m.p >250 °C. IR (KBr, ν/cm^{-1}):3459(OH), 1746, 1660, 1605 (2C=O, C=N).^{1}H NMR (DMSO-d6) δ ppm 0.6-1.8 (m, 10H, H-aliph), 7.8-8.1 (m, 5H, H-Ar,NH) and 11.5(s, 1H, COOH). ^{13}C-NMR (DMSO-d6): δ ppm 18.6-74.23, 124.8, 125.14, 127.16, 128.8, 132.6, 136.2 and 163.44. MS (EI): (m/z, %), 272 (M^+, 25).

1-((Dicyclopropylmethylene)amino)-1H-pyrrole-2,5-dione (17)

Brown crystals (from methanol) 55 %, m.p >260 °C. IR (KBr, ν/cm^{-1}): 1739, 1634 (2C=O, C=N).^{1}H NMR (DMSO-d6) δ ppm 0.8-2.1 (m, 10H, H-aliph), 6.2 (dd, 2H, H-Ar). ^{13}C-NMR (DMSO-d6): δ ppm 38.9, 20.4, 20.6, 21.08, 29.04, 131.65, 166.85 and 172.06. MS (EI): (m/z, %), 204 (M^+, 56).

1-((Dicyclopropylmethylene)amino)pyrrolidine-2,5-dione (18)

White needles (from DMF) 82 %, m.p 245-8 ºC IR (KBr, ν/cm^{-1}): 1735 (2C=O).^{1}H NMR (DMSO-d6) δ ppm 1.8-3.5 (m, 14H, H-aliph). ^{13}C-NMR (DMSO-d6): δ ppm 26.3, 172.0 and 172.2. MS (EI): (m/z, %), 206 (M^+, 62).

5-Benzylidene-3-((dicyclopropylmethylene)amino)-2-methyl-3,5-dihydro-4H-imidazol-4-one(19a)

Brown crystals (from ethanol) 62 %, m.p 152-154 °C. IR (KBr, ν/cm^{-1}): 1704, 1645, 1602 (C=O, 2C=N).^{1}H NMR (DMSO-d6) δ ppm 1.9-2.0 (m, 13H, H-aliph, CH_3) and 6.9-8.3 (m, 6H, H-Ar). ^{13}C-NMR (DMSO-d6): δ ppm 10.0, 14.04, 20.50, 21.06, 112.2, 130.4, 132.4, 136.9, 141.15, 159.34, 169.12 and 172.03. MS (EI): (m/z, %), 289 (M^+- 4, 10.6).

5-Benzylidene-3-((dicyclopropylmethylene)amino)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (19b)

Yellow crystals (from acetic acid) 74 %, m.p 158-160 °C. IR (KBr, ν/cm^{-1}): 1793,1725, 1653 (C=O,2 C=N).^{1}H NMR (DMSO-d6) δ ppm 0.8-2.1 (m, 10H, H-aliph) and 7.4-8.3(m, 11H, H-Ar,CH=C).^{13}C-NMR (DMSO-d6): δ ppm 10-20,

125.1-133.7 Ar-C, 163.06 and 166.9 MS (EI): (m/z, %), 352 (M^+, 1.9).

3-((Dicyclopropylmethylene)amino)-2-methylquinazolin-4(3H)-one (20)

Faint yellow needles (from ethanol) 82 %, m.p 230-232 °C IR (KBr, ν/cm^{-1}): 1675, 1660 (C=O, C=N).^{1}H NMR (DMSO-d6) δ ppm 1.02-2.1 (m, 13H, H-aliph, CH_3) and 7.9-8.2 (m, 4H, H-Ar). MS (EI): (m/z, %), 267 (M^+, 3.6).

Synthesis of 2,2-dicyclopropyl-3,4-dihydro-2H-naphtho[2,1-e][1,3,4]oxadiazine-5,6-dione (22)

2,3-Epoxy-1,4-naphthoquinone (5 mmol) was added to a solution of **2** (5 mmol) in absolute ethanol. The reaction mixture was refluxed in water bath for 2 h. The precipitate that formed was filtered off. This solid was dissolved in distilled water then cooled to 0°c and dil. HCl was dropwise added to the solution until reached to PH 6. The crude product was filtered off, washed with water, dried, recrystallized from methanol **to** give buff crystals of 22 in 89 % yield, mp 185-186 °C.

IR (KBr, ν/cm^{-1}):3450 (2NH), 1687 (2C=O).^{1}H NMR (DMSO-d6) δ ppm 1.2-1.5 (m, 10H, H-aliph), 6.1(s, 1H, NH) and 7.7-8.01 (m, 1H, H-Ar). ^{13}C-NMR (DMSO-d6): δ ppm 16.6-77, 110.99, 125.3, 125.93, 130.56, 131.87, 134.47, 159.48, 181, 24 and184.73. MS (EI): (m/z, %), 296 (M^+, 1.5).

Biological activity

The following materials were used: Dulbecco's modified Eagles medium (DMEM) (Lonza, Belgium), a fetal bovine serum (FBS) (Lonza, Belgium), an antibiotic and antimycotic (penicillin-streptomycin) (Lonza, USA) sample, a Kreps ringer bicarbonate buffer (Sigma Aldrich, USA), a trypsin/EDTA 0.25 % (200 mg/L EDTA, 170,000 U trypsin/L) (Lonza, Belgium), a bovine serum albumin (BSA) (Hyclone, USA). , a phosphate buffer saline 1X (PBS) (Hyclone, USA), Trypan blue 0.4 % (Lonza, USA), an MTT assay kit (Lonza, USA), Ciprofloxacin (Sigma Aldrich, USA), Cisplatin (Hospira UK Ltd.).

MCF7 and normal cell lines were obtained from VACSERA, EGYPT. Candida Albicans Fungus was obtained from Regional Center of fungi, Al-Azhar University, Egypt. Gram +ve *Staphylococcus Aureus* and Gram –ve *E. Coli* were obtained from Department of Biotechnology, Faculty of Post Graduate Studies for Advanced Sciences, Beni-Suef University, Egypt.

Anticancer activity

The cells were cultured in DMEM medium at 37 ° C, 5 % CO_2 supplemented with 10 % fetal bovine serum (FBS), 100 U mL-1 penicillin and 100 µg mL^{-1} streptomycin. Cell viability was estimated using MTT assay Kit which based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye to violet formazan crystals by succinate dehydrogenase inside living cells mitochondria. The cells were seeded into 96-well plate with a concentration of 6000 cells/well and incubated for 24 h. The medium was discarded and various concentrations of the test compounds dissolved in DMEM were added to wells. After another incubation with the same conditions, the medium was discarded, and 100 µL MTT (2 mg mL^{-1}) was added and incubated for 3 h at 37 °C. The produced purple formazan crystals were dissolved in 50 µL of DMSO. The plate was then incubated for 15 min at 37 °C and the optical density was read at 570 nm with a reference wavelength 630 nm as using Stat fax Elisa plate reader (Stat fax, USA). Cisplatin (Hospira UK Ltd.) was used as positive control and DMSO was used as the solvent for compounds and its final concentration was less than 0.2%. IC50 and IS50 were calculated. All in vitro tests were performed in triplicates.

Antimicrobial and antifungal activity tests

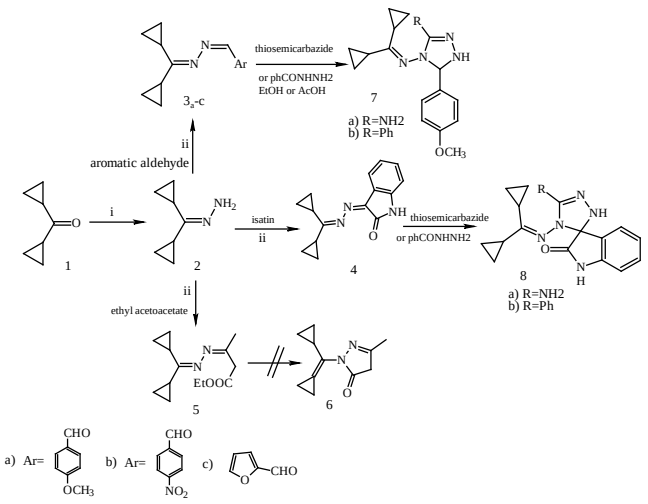
The studied compounds were diluted with DMSO to conc. 100 mg mL^{-1} then about 6mm diameter filter paper was soaked in 75 µl of diluted compounds. Ciprofloxacin (20 µg mL^{-1}) was used as positive control and DMSO was used as negative control. Nutrient agar plates were inoculated with Gram +ve *Staphylococcus Aureus*, Gram –ve *E. Coli* and *Candida Albicans* by sterile swabs and the tested compound containing discs were placed onto the nutrient agar surface by sterile forceps. Plates were incubated at 37°C/24 h. Inhibition zone was measured in mm. Tests were performed in triplicates.

Result and discussion

The target synthetic products **2–23** were prepared using synthetic procedures are summarized in Schemes 1-4. Condensation of dicyclopropyl ketone **1** with hydrazine hydrate in refluxing ethanol afforded the key hydrazone derivative (**2),** followed by condensation with appropriate aromatic aldehydes in refluxing ethanol formed the corresponding Schiff bases (**3-5)** in good yields. Similarly, the hydrazone **2** was condensed with isatin in the presence of a few drops of glacial acetic acid as a catalyst to produce the Schiff base **4** in a rather excellent yield. However, cyclization reaction of the hydrazine derivative **5** was unsuccessful.

The structures of the newly synthesized compounds **2-5** were confirmed on the basis of their spectral data (IR,^{1}H-NMR, ^{13}C-NMR and MS spectra). The infrared spectra of the formed hydrazones showed the absence of amino group and appearance of imino group at 1613-1659 cm^{-1}. In addition, ^{1}H-NMR spectrum of **3b** showed singlet signal at 3.79 ppm due to the methoxy group, while in the spectrum of compound **4** a singlet signal at 10.9 ppm for NH group was appeared. Also, the spectrum of compound **5** showed the presence of -$COOCH_2CH_3$ group, two protons appeared as a quartet at 4.0 ppm, three protons as a triplet at 1.22 ppm while, the three protons of the methyl group appeared as a singlet at 2.25 ppm.

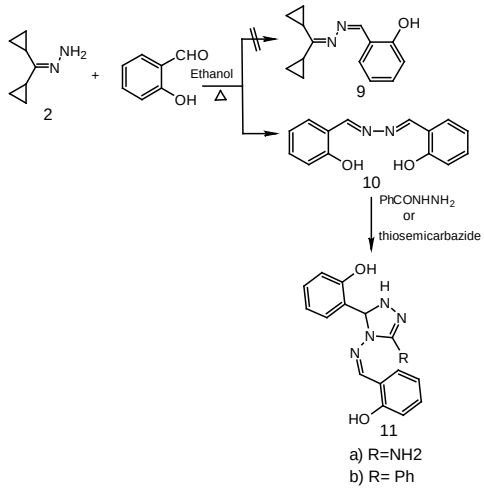
In view of the biological importance of triazoles,[23–27] our target was to synthesize new hydrazones attached to triazole moieties. Subsequent cycloaddition of imines **3a** and **4** with thiosemicarbazide or benzohydrazide in boiling ethanol in the presence of acetic acid furnished the triazole derivatives **7a, b** and **8a, b** respectively. Analytical and spectral data well characterized all the synthesized compounds.



Scheme 1. Synthetic route to obtain target hydrazone derivatives **3-5 (**(i) $NH_2NH_2.H_2O$, ethanol, reflux; (ii) ethanol, glacial CH_3COOH, reflux) and subsequent formation of triazole derivatives by reaction of the synthesized Schiff bases with thiosemicarbazide and benzohydrazide

The amino protons in the sepctra of compounds **7a** and **8a** were appeared in the range of 9.8-12.9 ppm. All the aromatic protons were found in the range of 6.7-8 ppm. IR spectrum for compound **8b** ascertained its structure by the appearance of the characteristic bands at 3438 and 1691 cm^{-1} for the amino and carbonyl groups. Its ^{1}H-NMR spectrum showed singlet signals at 12.4 and 11.0 ppm assigned to 2NH protons. The appearance of aromatic hydrogen signals was confirmed by the multiplet signals in the range of 6.8-7.6 ppm. The ^{13}C-NMR and mass spectra were in good agreement with the molecular formula. On the other hand, with an unexpected reaction of salicylaldehyde with hydrazone **2** it gave the light yellow crystals of salicylaldehyde azine **10** with 95 % yield which was synthesized previously.[28] The proposed mechanism accompanied by degradation of the hydrazone group and reformation of the starting dicyclopropyl ketone then condensation of the resulting salicylaldehyde hydrazone with another mole of salicylaldehyde. Comparison with the reported spectra and melting point data with the synthesized compound gave us absolute confidence to assign it as salicylaldehyde diazine **10**.

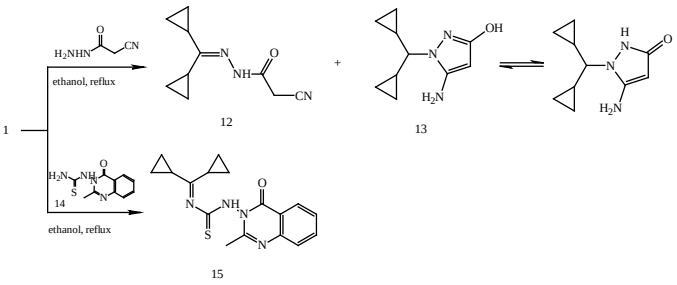
The spectral data of **10** were in good agreement with the expected structure of the compound. Its IR spectrum showed absorption bands at 3445 and 1622 cm^{-1} due to 2OH and 2C=N groups and absence of absorption band of NH_2 group. ^{1}H-NMR and ^{13}C-NMR revealed the absence of aliphatic protons and carbons of the cyclopropyl rings, respectively. Its mass spectrum exhibited a molecular ion peak at m\z 240 which consistent the molecular formula of the bis-azine **10.** We have attempted to prepare the Schiff base derivative **9** by the addition reaction of thiosemicarbazide and benzohydrazide with compound **2**. As the unexpected direction of the reaction led the formation of bis azine **10,** this compound was reacted with thiosemicarbazide and benzohydrazide to form triazoles **11a** and **11b**, respectively. Their structures were assigned by their spectral and analytical data. IR spectrum for **11a** showed the presence of NH_2 bands at 3173 and 3137 cm^{-1} and the mass and NMR spectra were confirmed the cyclization reaction with thiosemicarbazides. In the same way, the formation of **11b** could be confirmed by its spectral data.



Scheme 2. The reaction of hydrazine **2** with salicylaldehyde and subsequent with thiosemicarbazide and benzohydrazide

Compound **1** was reacted with cyanoacetic acid hydrazide in ethanol as a solvent given a mixture of cyanoacetohydrazone and pyrazole derivatives (**12** and **13)** which can exist in two tautomeric ketone-enol forms), respectively and these products were separated easily from ethanol by cooling. Treatment of dicyclopropyl ketone with thiourea derivative **(14)** in boiling ethanol gave the corresponding quinazoline derivative (**15)**.

The structures of the **12** and **13** were elucidated on the basis of their IR, ^{1}H-NMR, ^{13}C-NMR and MS spectra. For example, the IR spectrum of **12** revealed the presence of absorption band at 2264 cm^{-1} characteristic for cyano group. There were no found any absorption bands at the region of cyano group, but there was found absorption bands at 3166 due to the presence NH_2 group in the spectrum of compound **13**. NMR spectra also confirmed the structures with appearance of methylene signals at 3.9 ppm and NH_2 signal at 10.8 ppm. Further, the structure of compounds **12-15** was established on the basis of analytical and spectral data.

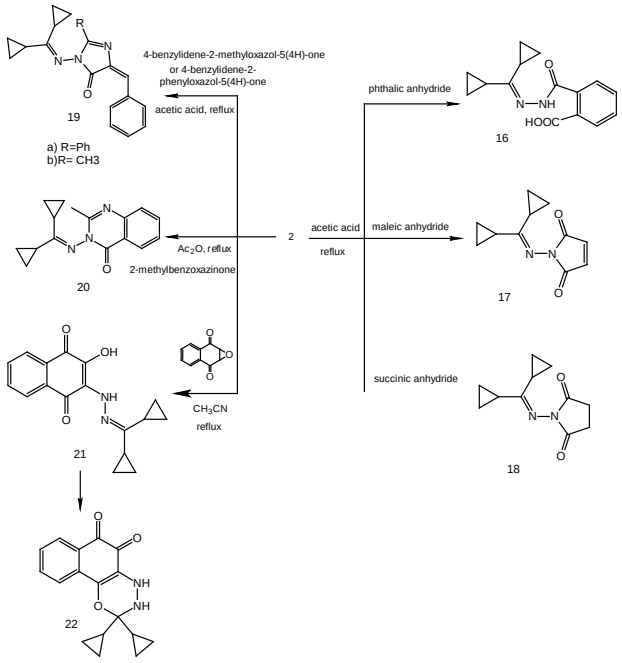


Scheme 3. reaction of dicyclopropyl ketone 1 with cyanoacetic acid hydrazide and thiourea derivative in absolute ethanol

Finally, the hydrazone derivative **2** was reacted with different anhydrides like phthalic, succinic and maleic anhydrides in refluxing acetic acid to give phtalamido (a ring opening of the anhydride was observed and attempts made to cyclize the synthesized product were failed), 1-succinimido and 1-pyrrole analogs **16-18**, respectively. The formation of compounds **16-18** could take place through the nucleophilic addition by the nitrogen nucleophile of the hydrazide on the carbonyl group of the anhydride leading to ring opening followed by cyclisation with the elimination of a water

molecule. The same methodology was extended to the preparation imidazolone derivatives **19a,b** by the reaction of **2** with appropriate oxazolones, respectively**.** Furthermore, the reaction of **2** with 2-methylbenzoxazinone gave quinazolinone **20** in 54 % yield. 2,2-Dicyclopropyl-3,4-dihydro-2H-naphtho[2,1-*e*][1,3,4]oxadiazine-5,6-dione (**22**) was prepared by condensation of (dicyclopropyl-methylene)hydrazone (**2**) with 2,3-epoxy-1,4-naphtho-quinone in boiling acetonitrile in excellent yield through the formation of intermediate **21** with subsequent cyclization.

Structural characterizations of the synthesized compounds **14-22** were performed by IR, ^{1}H and ^{13}C NMR and mass spectrometry. The IR spectra of compounds **17** showed the absence NH_2 absorption band and the presence of characteristic amide -C=O group stretching bands around 1739 cm^{-1}. IR of **16** showed a distinctive band at 1746 cm^{-1} due to the carbonyl of carboxylic group. Its ^{1}H-NMR spectrum showed the presence of aromatic protons and exhibits a singlet signal at 11.5 ppm due to proton of COOH group. Analysis of the ^{13}C-NMR spectra of the hydrazones **16-17** revealed the presence of a signal for the carbonyl group of amide groups at approximately 172 ppm. The mass spectra of compounds **16-17** revealed the presence of the molecular ion peaks. IR and NMR spectra confirmed the structures of **19a,b** and **20** by the disappearance of the NH_2 group signals besides appearance of the expected signals. The structure of the synthesized compound **22** was ascertained on the basis of its IR, mass and NMR spectral characteristics. The presence of NH and C=O functional groups was marked by the appearance of stretching bands at 3450 and 1678 cm^{-1} respectively. Its ^{1}H-NMR spectrum showed two singlet signals at 6.3 and 11.6 ppm characteristic for two NH protons and the carbonyl signals are appeared at 184.73 and 181.24 ppm in the ^{13}C-NMR spectrum.



Scheme 4. Reaction of hydrazone derivative 2 with anhydrides, oxazolone, benzoxazinone and epoxy-1,4-naphthoquinone derivatives

Antimicrobial activity

The newly synthesized compounds were evaluated for their antibacterial and antifungal activities against *Staphylococcus Aureus, Escherichia coli* and *Candida Albicans.* Ciprofloxacin was used as reference material for the comparison. The results for antimicrobial activity showed that compounds **10, 19a** and **20** exhibited higher bioactivity against *Staphylococcus* than the standard material and compounds **3b, 3c, 4, 11a, 11b, 12, 13, 15** and **7a** showed moderate activity and compounds **5, 16** and **8b** possessed excellent antibacterial activity against *E. Coli*. Most of the synthesized compounds showed high to good antifungal action against *C. albicans* growth in comparison with the reference material. Results showed that compounds **3b, 3c, 15** and **7a** were more active among all the test compounds followed by compound **9b** and **18**. The synthesized compounds **4, 12, 19b** and **20** did not show any antifungal activity at the studied concentrations against *C. albicans* growth.

Table 1: Antimicrobial activity of the synthesized compounds

Compounds	**Antimicrobial activity inhibition zone in mm**		
	Staphylococcus Aureus	***E. Coli***	***Candida albicans***
Ciprofloxacin	12	9	9
2	19	17	18
3a	15	21	15
3b	-	16	25
3c	16	12	22
4	18	-	-
5	-	25	15
7a	19	22	22
7b	10	18	12
8a	10	15	13
8b	-	24	18
10	24	15	18
11a	16	10	15
11b	17	22	20
12	16	22	-
13	15	19	9
15	17	22	15
16	-	24	21
18	10	-	12
19a	20	22	15
19b	-	-	-
20	21	15	-
22	11	18	10

Anticancer activity

The anticancer activity for the synthesized compounds was evaluated in vitro screening against breast cancer cell line (MCF-7) (Table 2). Cisplatin was used as the reference material. From the results of Table 2, it was found that compound **4** exhibited the most excellent anticancer activity for MCF-7 cell lines, with an IC_{50} value of 21.5 it proved to be much more active than the reference drug and compared to most of the tested compounds. This is may be due to the presence of indole moiety.

Furthermore, compounds **13, 18** and **19a** were found to be more active than the reference drug also with IC_{50} 22.4, 23.6 and 25.5, respectively. The cell killing potency of **13** is high and this may be due to the presence of two active free functional groups OH and NH_2 attached to pyrazole moiety. While the presence of imido and imidazolone skeletons and the presence of phenyl groups enhanced their cytotoxic activity of **18** and **19a** respectively. On the other hand, the other tested synthesized compounds were exhibited moderate to weak cytotoxic activity.

The selectivity index (SI) for our newly synthesized drugs was calculated by comparison the cytotoxic effect of drugs against cancer cell line with its cytotoxic effect against human lung fibroblast normal cell line MRC-**5** and the findings came with high satisfaction, where compounds **4, 13, 18, 19a** showed selectivity to cancer cell line higher than cisplatin selectivity, also compound **2** showed the same selectivity in comparison with cisplatin.

Moreover, compounds **3a, 3c, 7a, 7b, 8a, 11b, 12, 19b** showed a satisfactory selectivity index, especially **8a** that was closer to cisplatin selectivity index. These results proved that our newly synthesized hydrazone derivatives were selective and specific to hepatocellular carcinoma cell line HEPG2 more than normal human cell line.

Table 2. Cytotoxicity of cisplatin and the new compounds against MCF-7 breast cancer cells and normal cell line

MTT assay IC50 24h (µM)			***SI***
Compounds	**MCF7**	**MRC-5**	
Cisplatin	28.4	90	3.1
2	62.8	200	3.1
3a	50	120	2.4
3b	100	>200	-
3c	50.5	150	2.9
4	21.5	75	3.4
5	>100	150	-
7a	40.8	90	2.2
7b	51.7	120	2.3
8a	50	150	3
8b	84.2	100	1.1
10	90.6	150	1.6
11a	80.2	85.2	1.06
11b	45.2	100	2.2
12	55.8	150	2.6
13	22.4	80	3.5
15	48.6	>200	-
16	>100	95	-
17	100	150	1.5
18	23.6	100	3.2
19a	25.5	84.7	3.3
19b	75.4	175	2.3
20	50	75	1.5
22	>100	50	-

IC_{50}: drug concentration that inhibits cell growth by 50%. *SI* was calculated by dividing IC_{50} value of lung fibroblast normal cell line for each compound against IC_{50} of the cancer cell line**.**

Conclusion

Some new hydrazones based on (dicyclopropylmethylene)hydrazine **2** were synthesized and transformed into a series of heterocyclic products such as imidazolone, quinazolinone, triazole and indole derivatives. The antimicrobial and antitumor activities of the newly synthesized compounds were evaluated. Most of the compounds showed higher activity compared with Ciprofloxacin as positive controls and some of the compounds showed satisfactory activity against a breast cancer cell line (MCF7) compared with cisplatin as a positive control.

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