Reactivity of 1,3-diarylpropenones towards some nucleophilic reagents and screening of the biological activity of the products

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Abstract: Some novel chalcone derivatives **(1a-c)** were prepared by the reaction of 2-acetylpyridine, 4-bromoacetophenone with 4-N, N-dimethyl benzaldehyde, 2-chloro-, and/or 4-methoxy-benzaldehyde under Claisen-Schmidt conditions. The chalcones were then reacted with some nucleophiles such as urea derivatives to give substituted pyrimidines (2 and 5). They were subjected also to carbon nucleophiles such as ethyl acetoacetate to give 6-carbethoxy-3,5-diaryl cyclohexenone (9) which on treatment with thiosemicarbazide gave the triazolyl derivative (10). The new compounds were characterized by IR, ^{1}H-,^{13}C-NMR and Mass spectral data. Some of the new compounds were tested for their antimicrobial activity as well as antitumor and the results were encouraging.

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Keywords: Chalcone derivatives, pyrimidine, cyclohexene, pyrazolines, biological activity.

1. Introduction:

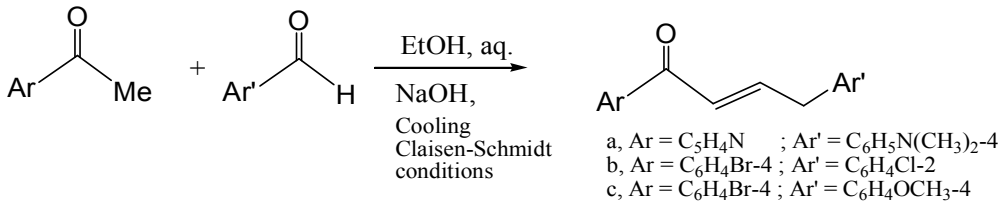
α,β-Unsaturated ketones, due to their unique stereo-electronic properties, are one of the most important substituents in organic chemistry. These derivatives form the central core for a variety of important biological compounds[1,2], which are known collectively as chalcones[3]. They show antibacterial[2], antifungal[4], antitumor[5], antioxidant[6,7] and anti-inflammatory properties[8,9].

They were usually prepared by an aldol condensation between aromatic, or heterocyclic aldehyde and an acetophenone derivative in a base catalyzed medium[10].

Because of the close Van-der-Waals radii, such compounds play a very important role in drug-receptor interactions. The >C=O group is highly polarized towards the electronegative oxygen atom and these results in a quite different electron-density distribution and significantly changes the reactivity of the molecule towards different nucleophiles.

α,β-Unsaturated carbonyl compounds are readily active derivatives. Their application in synthesis broadens the arsenal of existing building blocks and demonstrates the prespective and potential of this approach for the introduction of azo groups into compounds from different nucleopiles.

Antibacterial, antifungal as well as antitumor properties of some synthesized products are included in this study.



a, Ar = C_5H_4N ; Ar' = $C_6H_5N(CH_3)_2$-4
b, Ar = C_6H_4Br-4 ; Ar' = C_6H_4Cl-2
c, Ar = C_6H_4Br-4 ; Ar' = $C_6H_4OCH_3$-4 **(1)**

2. Experimental

Apparatus

IR spectra were run on a Perkin-Elmer 1724 FTIR instrument in KBr pellets. Characteristic wave numbers are given in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra were recorded on Varian EM-360L spectrometer using DMSO-d_6 as a solvent and TMS as internal standard. Elemental analysis were performed using PE2400 CHN analyzer. Melting points were measured in capillary tubes with a Galen Kamp apparatus and are uncorrected.

Chemistry

General procedure for the preparation of compounds 1a-c.

4-acetylpyridine and/or 4-bromoacetophenone (0.01 mol) and aromatic aldehydes namely, 4-N,N-dimethylaminobenzaldehyde, 2-chlorobenzaldehyde and/or 4-methoxy benzaldehyde (0.01 mol) were dissolved in ethanol (15ml), cooled and stirred well for ½hr., aqueous NaOH (10%, 5ml) was added and the reaction mixture was further stirred for 2 hrs while

cooling, then at room temperature for 1 hr. the reaction mixture was left on the refrigerator overnight. It was then filtered off and the product was washed well with dilute alcohol then recrystallized from absolute EtOH, c.f. Table 1.

1a ^{13}C-NMR (DMSO-d_6) δ39.9, 40.1ppm (2x3H, $2CH_3$); 114.1, 114.7 (2CH), 2x122.9 (2CH); δ123.1 (CH–C=O); 124.7, 124.8 (2CH), 125.8 (C-CH); 143.2 (C–C=O); 146.2 (CH–C); 150.1 (=C–N); 153.1, 153.6 (2xCH–N); 189.9(C=O).**1c** ^{13}C-NMR (DMSO-d_6). 60.1(CH_3O-); 2x114.3 (2xCH), 112.7 (CH=CH), 127.4, 127.8 (2xCH), 128.3 (C=CH) 128.9 (Br-C); 132.6, 132.9 (2xCH); 146.1 (CH=CH), 160.3 (=C–O); 190.3 (C=O).

General procedure for the preparation of compounds 2,5a,b

An equimolar mixture of **1a-c** and guanidine carbonate/or thiourea (0.01 mol) was heated under reflux in 30ml of absolute ethanol containing sodium ethoxide for 6 hrs. After cooling the product was filtered off, washed well with water and recrystallized to give **2** and **5a,b** respectively, c.f. Table 1.

2 ^{1}H-NMR (DMSO-d_6) at 1.2, 1.8 ppm (2xs, 2x3H, 2 xCH_3); 2.49, 2.5 (d, 2H, CH_2), 3.03 (t, 1H, CH), 6.11 – 8.72 (m, 10, Ar – H), 9.65 (s, 2H, NH_2).

5b ^{1}H-NMR (DMSO-d_6) at 2.3, 2.4 (2d, 2H, CH_2); 3.72 (s, 3H, OCH_3), 3.89 (t, 1H, CH), 5.36 (s, 1H, cyclic NH), 6.97 – 8.51 (m, 8H, Ar – H).

Formation of the 2-N(diazoaryl)-4,6-diaryl-1,3-pyrimidine derivatives 3a,b

To a well cooled suspension of 2-N-(pyrimidinium) diazonium chloride (prepared by diazotizing **2** (0.01 mol) with nitrous acid (0.01 mol) as usual, was added, drop-wise a well cooled solution of o-nitrophenol and/or β-naphthol (0.01 mol) in 5ml of aqueous sodium hydroxide and the mixture was stirred for 30min. at 0-5^{o}C then for 30min. at room temperature. It was then left overnight then filtered off and re-crystallized to give 3a,b; c.f. Table 1.

3a ^{1}H-NMR (DMSO-d_6) at 1.62, 1.64 (2xS, 2x3H, 2xCH_3); 2.61, 2.81 (2xd, 2H, CH_2), 3.72 (t, 1H, CH), 6.54–8.83 (11H, Ar–H), at 10.91 (s, 1H, OH). MS:M)$^{+\cdot}$ 445 (2.5%) for $C_{23}H_{23}N_7O_3$, $C_{12}H_{14}N_5$ for M)$^{+\cdot}$ 309 (1.48%), 253 (100%) for the M)$^{+\cdot}$ $C_{14}H_{15}N_5$, M)$^{+\bullet}$ at 173 (29.9%) for $C_8H_7N_5$, M)$^{+\cdot}$ 166 for $C_6H_4N_3O_3$ (12.05%) M)$^{+\cdot}$; 78 (74.2%) for C_5H_4N.

Formation of the Schiff's bases 4a,b

A mixture of **2** (0.01 mol) and aromatic aldehydes namely 2-chlorobenzaldehyde / or 4-methoxybenzaldehyde (0.01 mol) in 30ml of absolute ethanol was well-stirred for 2 hrs., then refluxed on a steam-bath for 1 hr. after cooling the product formed was collected, washed well with 0.005N aqueous sodium hydroxide then dilute ethanol and recrystallized to give 4a,b, c.f. Table 1.

4a ^{1}H-NMR (DMSO-d_6); δ ppm 3.34 (2xs, 2x3H, $2CH_3$), 2.76, 2.91 (2d, 2H, CH_2), 3.71 (t, 1H, CH), 4.95 (s, 1H, CH), 6.72 – 8.19 (m, 12H, Ar – H). MS: 417.2 (53.61%) M)$^{+\cdot}$ for $C_{24}H_{24}N_5Cl$, M)$^{+\cdot}$ 216.5 (42.2%) for $C_{11}H_7N_3Cl$; 183 (100%) for $C_{10}H_8N_4$; M)$^{+\cdot}$ $C_8H_{12}N$ 122 (14.5%). 4b MS: 412 $(M-1)^+$ (1.67%), M)$^{+\cdot}$ at 316 (1.62%), M)$^{+\cdot}$ at 306 (4.4%), M)$^{+\cdot}$ at 185 (1.5%), M)$^{+\cdot}$ 159 (8.6%), 145 (100%), M)$^{+\cdot}$ 135 (43.32%), M)$^{+\cdot}$ 106 (6.1%), M) 77 (12.0%).

Hydrazinolysis of 5. formation of 2-hydrazino-4, 6-diaryl 2(1H) pyrimidine (6).

A mixture of **5b** (0.01 mol) and hydrazine hydrate (0.03 mol) in 30ml of absolute ethanol was refluxed for 12 hrs. after concentration and cooling, the product was collected, washed well with dilute ethanol and re-crystallized to give **6**; c.f. Table 1.

6 ^{1}H-NMR (DMSO-d_6) at δ 2.58, 2.59 (2xd, 2H, CH_2), δ 3.44 (s, 3H, OCH_3), δ 3.51, 3.53, 3.56 (t, 1H, CH), δ 4.41, 4.43 (d, 1H, cyclic NH), δ 7.18 – 8.13 (m, 8H, Ar–H), at δ 8.34 (s, 2H, NH_2).

Reaction of 5 with copper bronze. Formation of the disulphanyl pyrimidine-derivative (7)

To a suspension of **5b** (0.01 mol) in 20ml of dry xylene was added 1g of copper bronze and the mixture was heated under reflux for 10 hrs., filtered off and the filtrate was evaporated under reduced pressure. The product was treated with pet. ether (b.p. 60–80^{o}C) then re-crystallized to give 7, c.f. Table 1. **7** ^{1}H-NMR (DMSO-d_6): δ 1.92, 2,01, 2.02; 2.03 (2d, 2xH_2, 2xCH_2), δ 3.7, 3.71 (2xt, 2xH, 2xCH); 3.73, 3.41 (2xs, 2x3H, 2xOCH_3), 6.72–8.31 (m, 16H, Ar–H). MS: 748 M)$^{+\cdot}$ for $C_{34}H_{28}N_4O_2S_2Br_2$ and the molecular ion peaks at m/e 448 (4.6%), 408 (11.02%), 224 (6.14%), 157 (100%), 146 (9.4%).

Reaction of 5 with anthranilic acid. Formation of the pyrimido [1,2-a] quinazolin-6-one derivative (8).

An equimolar mixture of **5b** and anthranilic acid (0.01 mol) was heated under reflux on a sand-bath (keeping the temperature) at 170 – 185^{o}C for 3 hrs. After cooling it was diluted with water and the residue was collected washed well with dilute alcohol and recrystallized to give **8** (c.f. Table 1). 8 ^{1}H-NMR (DMSO-d_6): δ 3.73 (s, 3H, OCH_3), 2.02, 2.029 (2d, 2H, CH_2), 2.13, 2.134, 2.139 (t, 1H, CH), 6.73 – 7.39 (m, 12H, Ar–H). ^{13}C-NMR (DMSO-d_6): δ 39.9 (CH_2), 56.7 (OCH_3), 64.9 (CH–CH_2), 114.1, 117.9, 122.3 (3xCH), 131.8, 131.9, 132.3, (3xCH), 133.7, 133.8, 144 (3xC=), 152.4 (C – Br), 163.3, 164.1 (2xC=N), 165.3 (C – O), 168.7 (C = O).

Reaction of 1a with acetoacetic ester. Formation of 5-(4-(dimethyl-amino) phenyl)-3-(pyridin-4-yl) cyclohex-2ene-1one-6-ethyl carboxylate derivative (9).

To a suspension of **1a** (0.01 mol) in 30ml of absolute ethanol containing (0.01 mol) of sodium ethoxide (prepared by dissolving 0.2g of sodium metal in 5ml of absolute ethanol was added ethyl aceto acetate (0.01 mol) and the mixture was heated under reflux for 6 hrs. after cooling it was diluted with water and the product was filtered off, washed well with water then dilute alcohol and re-crystallized to give **9**, c.f. Table 1. **9** ^{1}H-NMR (DMSO-d_6)**:** 1.39, 1.42, 1.46 (t, 3H, $\underline{CH_3}CH_2$), 2.14 – 2.25 (9, 2H, $\underline{CH_2}CH_3$), 2.38–2.51 (2d, 2H, CH_2), 2.85, 2.86 (2xs, 2x3H, 2xCH_3N), 6.39 (s, 1H, CH), 6.59 – 8.72 (m, 8H, Ar–H). MS; molecular ion $M)^{+\cdot}$ at m/e 364 (0.92%), molecular ion at 214 (1.96%), 166 (13.2%), 121 (42.1%), 79 (11.3%), 64 (9.2%), 55 (100%).

Reaction of 9 with thiosemicarbazide Formation of 5-(4-(dimethyl-amino)phenyl)-3-(pyridine-4-yl)- 6-(5'-thioxo-4',5'-dihydro-1H-1',2',4'-triazol-3'-yl) cyclohex-2-ene-1-one (10).

A mixture of **9** (0.01 mol), thiosemicarbazide (0.01 mol) and sodium acetate (0.005 mol) (dissolved in 1ml of water) in 30ml of absolute ethanol was refluxed for 6 hrs. The product was collected, washed well with dilute alcohol and re-crystallized to give **10**, c.f. Table 1. **10** ^{1}H-NMR (DMSO-d_6): 2.33, 2.34 (2xs, 2x3H, 2xCH_3), 2.33, 2.44 (2Xd, 2H, CH_2), 2.85, 2.86, 2.87 (t, 1H, CH), 4.19 (s, 1H, NH), 5.12 (s, 1H, NH), 6.47 (s, 1H, CH), 6.72–8.39 (m, 8H, Ar–H). ^{13}C-NMR (DMSO-d_6): δ 24.3 (CH), 36.3 (CH_2), 44.1, 44.13 (2xCH_3), 114.7, 114.8 (2xCH), 121.3, 121.4 (2xCH), 127.7, 128.3 (2xCH),, 136.7 (CH), 139.4 (C–CH), 144.7 (C–C), 149.9 (CH = N), 150.2 (C–N), 155.3 (C–C=N), 188.1 (C=S), 197.9 (C=O).

Reaction of 1a with hydrazine hydrate. Formation of 5-(N,N-dimethylphenyl)-4-(3-(pyridin-4-y)-4,5-dihydro-1Hpyrazol-5-yl (11).

A mixture of **1a** (0.01 mol) and hydrazine hydrate (0.01 mol) in 30ml of absolute ethanol was heated under reflux for 6 hrs. After concentration and cooling the product was collected, washed with dilute alcohol and re-crystallized to give **11**, c.f. Table 1. **11** ^{1}H-NMR (DMSO-d_6): δ 1.39, 1.40 (2xs, 2x3H, 2xCH_3), 2.01, 2.03 (2xd, 2H, CH_2), 2.81, 2.82, 2.83 (t, 1H, CH), 6.54 – 8.29 (m, 8H, Ar–H) 8.83 (s, 1H, NH). MS: molecular ion peak at m/e 266.13 (1.98%) $C_{16}H_{18}N_4$ and other molecular ion peaks at m/e 189 (11.3%), 121 for $C_8H_{11}N$ (13.3%), m/e 119 (1.71%) for C_6H_5N, m/e 106 (8.72%) for C_7H_8N**,** m/e 104 (2.51%) for $C_6H_4N_2$, m/e 81 (10.01%) for C_5H_7N, m/e 78 (45.1%), 77 (23.3%), 70 (11.3%) for $C_3H_6N_2$, m/e 67.17 (100%) for C_4H_5N.

Reaction of 11 with phenyl isothiocynate. Formation of N-(5-(4-dimethylamino)phenyl)-3-(pyridine-4-yl)-4,5-dihydropyrazol-1-yl)-phenylthioamide (12).

A mixture of **11** (0.01 mol) and phenyl isothiocyanate in 20ml of petroleum ether (b.p 60-80^{o}C) was refluxed for 6 hrs. After cooling the product was collected washed well with dilute alcohol and recrystallized to give **12** c.f. Table 1. 12 ^{1}H-NMR (DMSO-d_6): δ 2.39, 2.41 (2xs, 2x3H, 2xCH_3), 3.18, 3, 3.21 (2xd, 2H, CH_2), 3.3, 3.41, 3.49 (t, 1H, CH), 6.67 – 8.36 (m, 13H, Ar – H), 10.19 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): δ 38.5 (CH_2), 42.1, 42.2 (2xCH_3), 49.8 (CH-), 114.4, 114.7 (2CH), (115.3, 115.4 (2xCH), 117.3, 117.4 (2xCH), 121.3, 121.4 (2xCH), 124.7 (CH), 127.4, 127.7 (2xCH), 133.3 (C – C – N), 138.9 (C – C =N), 143.4 (C – NH), 151.2, 151.3 (2xN – CH), 153.2 (C = N), 154.1 (C – N), 179.1 (N – C = S).

3. Results and discussion

All the synthesized compounds were characterized by TLC, melting point, elemental analysis, IR, ^{1}H-, ^{13}C-NMR and mass spectra. The present study focuses on the synthesis of 1,3-diarly-2-propen-1-ones (1a-c). Compound **1a** reported in this paper is novel while compounds **(1b,c)** has been reported previously [(11)].

Thus, treatment of 1a with guanidine carbonate in ethanolic sodium ethoxide [(12)] affected cyclization to the corresponding 2-amino 4,6-diaryl-4,5-dihydropyrimidine. The IR of **2** revealed the presence of υNH_2/NH at 3264, 3334 cm^{-1}, υ1622 cm^{-1} for υC=N and devoid of any absorption for υC=O. The ^{1}H-NMR agreed well with the proposed structure as the singlet at δ 9.65 ppm of the two protons of NH_2, the doublet at δ 2.49, 2.50 ppm for the two protons at position 4 of the pyrimidine ring and the triplets at 3.03, 3.04, 3.05 ppm for the proton at C-5 indicating the presence of –CH_2–CH– in the structure. Compound **2** was reacted with nitrous acid at 0-5^{o}C to give the aryldiazonium chloride which was coupled with *O*-nitrophenol and/or -2-naphthol to give the corresponding 2-N-diazoaryl-4,6-diaryl 4,5-dihydro-1,3-pyrimidine derivatives **(3a,b).** The infrared spectrum of **3a** showed the –N=N– absorption band at 1577 cm^{-1} and the NO_2 asymmetrical absorption at 1661 cm^{-1}. Further confirmation of the structure was given by mass spectrum which showed M^+ at 445 and the M^+ at 253 as base peak. The Schiff's bases **(4a,b)** were synthesized by the interaction of **2** with O-chloro- and/or 4-methoxy benzaldehyde using ethanol as a solvent. The infrared spectra of **4a,b** showed the azomethine stretching absorptions at 1680, 1678 cm^{-1} and the bands at 1610, 1617 cm^{-1} assigned to C=N. In the ^{1}H-NMR spectrum of **4a** the presence of the pyrimidine moiety was manifested by two doublets of

doublets at δ 2.76, 2.91 and a triplet at δ 3.71. The N=CH proton appeared as a singlet at δ 4.95 and the twelve aromatic protons were discernible as multiplets in the range δ 6.72 – 8.19. The six $N(CH_3)$ protons were present as sharp singlet at 3.34. Further evidence of the structure was given by mass spectrometry, which showed M^+ ion peak at 417.2 and M^+ at 145 as a base peak.

The primidine thione derivatives **(5a,b)** were obtained from the interaction of 1b,c with thiourea in acidic medium in a polar solvent – EtOH (Scheme 1)[12,13]. The infrared spectra of **5a,b** showed the NH absorption at the region 3369, 3382 cm^{-1}, sharp absorption bands at 1623, 1607 cm^{-1} was assigned to C = N bond, bonds at 1224, 1221 cm^{-1} for the absorption band of C = S and a weak band around 2615 and 2551 cm^{-1} for the SH band arising due to thione ↔ thiol dynamic equilibrium. The ^{1}H-NMR of **5a** showed two doublets of CH_2 at δ 2.3, 2.4 and a triplet at δ 3.76 for the CH proton, a singlet at 5.49 ppm for the cyclic NH proton and the aromatic protons of the 4-bromophenyl and 2-chlorophenyl moiety appeared as a multiplet in the region 7.05 – 8.19 ppm which confirm the formation of the compound. Hydrazinolysis of **5b** yielded the corresponding hydrazone derivative **6**. The disulphide pyrimidine derivative **(7)** was obtained by refluxing 5b with copper bronze in a non-polar solvent such as xylene. The structure of 7 was confirmed by IR spectrum which revealed absorption at 3083, 2919, 2846 cm^{-1} for CH stretching, at 1609 cm^{-1} for the C = N stretching, at 1596 cm^{-1} for the aryl C=C stretching and an absorption at 452 cm^{-1} for the –S–S– with a band at 523 cm^{-1} for C–Br stretching. The mass spectrum of **7** agreed well with the propsed structure, it showed $M)^{+\cdot}$ at m/e 748.3 (2.3%) for $C_{37}H_{28}O_2S_2N_4Br_2$ and the base peak at m/e 157 (100%). The reaction of **5b** with anthranilic acid under solvent free conditions[14] affected cyclization to another important heteroaryl identified as pyrimido [**1,2-a**] quinazolin-6-one derivative **(8)**. The infrared spectrum of **8** has shown the carbonyl absorption band at 1661 cm^{-1} of quinazolinone moiety, a sharp absorption band at 1618 cm^{-1} for C=N stretching and a band at 1593cm^{-1} assigned for carbon-carbon double bond stretching absorption. The ^{1}H-NMR spectrum revealed the doublets at δ 2.02, 2.025, 2.027 and 2.029 ppm for the methylene protons, triplet at δ 2.13, 2.139 ppm for the methine proton, the singlet at δ 3.735 for the three protons of the methoxy group protons and the multiplet at δ 6.73 – 7.39 for the 12 protons of the aromatic moieties. ^{13}C-NMR spectrum showed the methylene moiety at δ 39.9, the aromatic methoxy moiety at δ 56.7, the methine group at δ 64.9, the aromatic moiety in the region δ 114.1–144 ppm, the C–Br at δ 152.4 ppm, the two C=N groups at δ 163.3, 164.1 and the C–O and the C=O singlets at δ 165.3 and 168.7 ppm respectively. 6-carbethoxy-3,5-diarylcyclohexenone derivative **(9)** was obtained by the Knoevenagel condensation[15] of ethyl acetoacetate and 1,3-diaryl-2-propen-1-one **(1a).** The IR spectrum of **9** showed two absorptions at 1693, 1665 cm^{-1} for the stretching of the two C=O groups, a band at 1596cm^{-1} for the absorption of C=C, the absorption bands at 3080, 2928 and 2880 for the C–H stretching and the overtone of the CH_3 bending vibration at 1370.1 cm^{-1}. The strong stretching absorption at 1266 cm^{-1} was assigned to the C–N band of the pyridine nucleus. The ^{1}H-NMR spectrum of **9** showed a triplet at 1.39, 1.42, 1.46 for the methyl protons of the ester, a quartet at 2.14 – 2.25 ppm for the methylene protons of the ester, two doublets at 2.38, 2.39; 2.48, 2.51 ppm for the CH_2 in the cyclohexene moiety; at 2.85; 2.86 two singlets for each three protons of the $N(CH_3)_2$, at δ 6.39 a singlet for the CH proton and the aromatic protons were showed as a multiplet of 8 at δ 6.59 – 8.72 ppm. The mass spectrum of **9** showed the $M)^{+\cdot}$ at 364 (0.9%) and the base peak at m/e 55 (100%).

The cyclocondensation of **9** with thiosemicarbazide in EtOH afforded the 5-thioxotriazolyl cyclohexenone derivative **(10)**. The IR spectrum of **10** revealed the C=O stretching band at 1676 cm^{-1}, the stretching band at 3259 cm^{-1} for NH absorption, 3066, 2988, 2828 cm^{-1} were the stretching bands for the CH absorption, a stretching band at 1611 cm^{-1} for the C = N absorption, a stretching band at 1595 cm^{-1} for the C = C absorption, at 1223.7 cm^{-1} a band assigned for the C = S absorption with a weak band at 2562 cm^{-1} for the stretching absorption of SH. The ^{1}H-NMR showed signals at δ 2.33, 2.34 as two singlets for the two CH_3 groups of $N(CH_3)_2$, two doublets at δ 2.33, 2.44 for the CH_2 protons and a triplet at 2.85, 2.86, 2.87 for the CH proton, at δ 4.19 and 5.12 ppm two singlets for the (NH–C=S) proton and NH–C=S proton in the heteroaryl moiety, at 6.47 a singlet for the (CH–C=O) proton and the eight aromatic protons appeared as a multiplet at δ 6.72–8.39 ppm.

With the objective of synthesizing pyrazoline containing pyridine moiety heterochalcone, **(1a)** was treated with hydrazine hydrate in ethanol to give **11** in considerable yield (66%) within 6 hrs. The IR spectrum of **11** showed a broad absorption band at 3255 cm^{-1} indicating the NH group of the pyrazoline moiety, with devoid of the absorption of the carbonyl moiety and the 1633 cm^{-1} was assigned to C=N group of the pyrazoline moiety. Further evidence of the structure was given by the mass spectrometry, which showed the $M)^{+\cdot}$ ion peak at 266.13 (1.98%) and the

base peak at m/e 67.17 (100%). Moreover, the reaction of **11** with active olefinic compounds, such as phenyl isothiocyanate was investigated. Thus, the reaction of 3,5-diaryl-4,5-dihydropyrazole **(11)** with phenyl isothiocyanate in pet. ether (b.p. 60-80^{o}C) afforded the corresponding 3,5-diaryl-4,5-dihydropyrazol-1N-phenylthioamide **(12).** The IR spectrum of **12** revealed the presence of NH stretching absorption at 3310 cm^{-1}, the presence of CH stretching absorption at 3310, 2982, 2828 cm^{-1}, the presence of C = N stretching absorption at 1609 cm^{-1}, the presence of C=C stretching absorption at 1590 cm^{-1} and the C=S–NH stretching absorption at 1221 cm^{-1}. The ^{1}H-NMR showed signals at δ 2.39, 2.41 ppm as two singlets for the two N–CH_3, two doublets at δ 3.18, 3.21 ppm for the two protons of the CH_2, a triplet at δ 3.30, 3.41, 3.49 ppm for the methine proton, a multiplet at δ 6.67–8.36 ppm for the 13-aromatic protons and a singlet at δ 10.19 for the NH–C=S proton. Further evidence was given by ^{13}C-NMR which showed the methylene group at δ 38.5, the two methyl groups of $N(CH_3)_2$ at δ 42.1, 42.2, the methine group at 49.8 and the aromatic moiety at δ 114.4–127.7. The C–N signal at δ 133.3, the C=N at δ 138.9, the C–NH at δ 143.4 the C = N at δ 153.2 and the N–C=S at 179.1 ppm.

Table 1. Characteristic data and IR spectra of compounds 1-12

Comp. No.	M.P.(°C) yield%	Solvent of a crystallization colour of crystals	Molecular formula (Mol. Wt.)	Analysis calcd./found%					υ(cm^{-1})									
				C	H	N	Cl	Br	C=S	OH	NH	C=O	C-H	C=N	C=C	C–Cl	C-Br	–N=N–
1a	122-124 77	EtOH Orange	$C_{16}H_{16}N_2O$ (252)	76.19 76.2	6.34 6.3	11.11 11.1	-- --	-- --	--	--		1667	3080 2910 2828	1605	1580	--		--
1b	106-162 58.2	EtOH Pale yellow	$C_{15}H_{10}ClBrO$ (321.5)	55.98 56.00	3.11 3.10	-- --	11.04 11.10	24.88 24.90	--	--		1682	3020 2918 2889	1603	1576	560	505	--
1c	128-138 48.6	EtOH White	$C_{16}H_{13}BrO_2$ (317)	60.56 60.60	4.10 4.10	-- --	-- --	25.23 25.3	--	--		1665	3022 2975 2880	1611	1590	--	535	--
2	130-132 80	Pet. Ether Brown	$C_{17}H_{21}N_5$ (295)	69.15 69.20	7.11 7.10	23.72 23.70	-- --	-- --	--		3264 3334	--	3033 2919 2888	1622	1599	--		--
3a	158-160 60	EtOH Light brown	$C_{23}H_{23}N_7O_3$ (445)	62.022 62.10	5.16 5.20	22.022 22.10	--	-- --		3444	3233	--	3069 3083 2828	1605	1569	--		1577
3b	171-172 74	EtOH Dark orange	$C_{27}H_{27}N_6O$ (451)	71.84 71.80	5.98 5.90	15.52 15.50	-- --	-- --		3446	3372	--	3085 3009 2958	1604	1567	--		1573
4a	128-130 80	Pet. Ether Dark brown	$C_{24}H_{22}N_5Cl$ (415.5)	69.31 69.30	5.29 5.30	16.84 16.80	5.84 8.50	-- --				--	3150 3010 2988 2822	1610	1591	571		
4b	150-152 71	Pet. Ether Brown	$C_{25}H_{25}N_5O$ (411)	72.99 73.00	6.08 6.10	17.03 16.90	-- --	-- --				--	3091 2989 2822	1617	1586	--		
5a	184-186 70	EtOH Deep yellow	$C_{16}H_{12}N_2SClBr$ (379.5)	50.59 50.60	3.16 3.20	7.37 7.40	9.35 9.40	21.08 21.10	1224		3232		3080 2920	1623	1601	619	505	
5b	190-192 66	EtOH Pale. Yellow	$C_{17}H_{15}N_2OS^*Br$ (375)	54.40 54.40	4.00 4.00	7.46 7.50	-- --	21.33 21.50	1221	--	3382	--	3006 2988 2828	1607	1580	--	490	--
6	70-72 70%	Pet.ether Yellow	$C_{17}H_{17}N_4OBr$ (373)	54.69 54.70	4.55 4.60	15.01 15.10	-- --	21.44 21.50	--	--	3255	--	3170 2982 2818	1606	1586	--	499	--
7	228-230 72	EtOH Yellow	$C_{34}H_{28}N_4O_2S_2^*Br_2$ (748)	54.54 54.60	3.74 3.70	7.48 7.50	-- --	21.39 21.38	--	--	--	--	3083 2919 2896	1609	1596	--	523	--
8	170-172 60	Pet/ ether Pale yellow	$C_{24}H_{18}N_3O_2Br$ (460)	62.608 62.70	3.91 3.90	9.13 9.20	-- --	17.39 17.40	--	--	--	1661	3132 2929 2842	1616	1593	--	489	
9	146-148 80	Pet. Ether Deep brown	$C_{22}H_{24}N_2O_3$ (364)	72.57 72.60	6.95 7.00	7.69 7.70	-- --	-- --	---	--	--	1693 1665	3080 2928 2880	--	1596	--	--	--
10	134-136 55	EtOH Brown	$C_{21}H_{21}N_5OS^*$ (391)	64.45 64.50	5.37 5.70	17.902 17.90	-- --	-- --	1223.7	--	3259	1676	3066 2988 2828	1611	1595	--	--	--
11	140-142 66	EtOH Yellow	$C_{16}H_{18}N_4$ (266)	72.18 72.20	6.76 6.80	21.052 21.10	-- --	-- --	--	--	3255	--	3006 2928 2888	1633	1590	--	--	--
12	160-162 62	Pet ether Dark orange	$C_{23}H_{23}N_5S^*$ (401)	68.82 68.80	5.73 5.70	17.45 17.50	-- --	-- --	1221	--	3310	--	3130 2982 2828	1609	1590	--	--	--

Where [a]pet. ether = petroleum ether, b.p 60-80 ^{o}C ; b = Micro analytical results for sulfur are 8.4 for 5a, 8.6 for 5b and 7, 8.2 for 10, and 8 for 12

Antibacterial studies:

The newly synthesized compounds were screened for their antibacterial activity against four fungi namely, *Aspergillus fumigatus* (RCMB 02568), *Syncephalstrum racemosum* (RCMB 05922), *Geotricum candidum* (RCMB 05097), *Candida albicans* (RCMB 05036); against Gram-positive bacteria namely, *Streptococcus pneumoniae* (RCMB 010010), *Bacillis subtilis* (RCMB 010067) and against Gram-negative bacteria namely, *Pseudomonas aeruginosa* (RCMB 010043) and *Escherichia coli* (RCMB 010052) bacterial strains by disk diffusion method[17,18]. A standard inoculums (1-2x10^7 c.f. 4/ml 0.5 McFarland standards) was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. The disks measuring 6mm in diameter were prepared from Whatman no.1 filter paper and sterilized by dry heat at 140°C for 1h. The sterile disks previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. Amphotericin B was used as standard control for the fungi; Ampicillin was used as standard control for the Gram-positive bacteria and Gentamicin was used as standard control for the Gram-negative bacteria, while the disk poured in DMSO was used as negative control. The plates were inverted and incubated for 24hrs. at 37°C. The susceptibility was assessed on the basis of diameter of zone inhibition against fungi,. Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the control. The bacterial zones of inhibition values are given in Tables 2,3.

Table 2. Antibacterial activity of compounds (1b, 1c, 5b, 6-8).

Comp. No.	Diameter of zone of inhibition (mm)			
	Gram-positive bacteria		Gram-negative bacteria	
	St.pneumoniae	***B.subtilis***	***P.aeruginose***	***E.coli***
1b	13.7 ± 0.35	16.3 ± 0.58	NA	13.7 ± 0.35
1c	16.2 ± 0.35	17.1 ±0.58	NA	14.6 ± 0.44
5b	15.8 ± 0.62	18.2 ± 0.35	NA	14.9 ± 0.58
6	21.4 ± 0.44	26.8 ± 0.58	NA	16.7 ± 0.58
7	11.4 ± 0.44	13.6 ± 0.63	NA	10.4 ± 0.29
8	18.6 ± 0.58	20.8 ± 0.35	NA	16.9 ± 0.58

Standard (positive) controls are *Ampicillin* and Gentamicin and negative control (DMSO) measured by the Halo Zone Test (unit, mm).

Table 3. MIC and MBC of compounds (1b, 1c, 5b, 6-8)

Comp. No.	Diameter of zone of inhibition (mm)							
	Gram-positive bacteria				Gram-negative bacteria			
	St.pneumoniae		***B.subtilis***		***P.aeruginose***		***E.coli***	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1b	100	100	50	100	>100	>100	100	100
1c	50	100	25	50	>100	>100	50	100
5b	50	100	25	25	>100	>100	50	100
6	125	50	12.5	25	>100	>100	25	50
7	100	100	100	100	>100	>100	100	100
8	25	100	25	50	>100	>100	50	50
Standard	12.5	50	12.5	50	12.5	50	12.5	50

MIC (µg/mL) = minimum inhibitory concentration, that is the lowest concentration of the compound to inhibit the growth of bacteria completely.

MBC (µg/mL) = minimum bacterial concentration, that is the lowest concentration of the compound for killing the bacteria completely, positive control is Ampicillin and Gentamicin.

Compound **(6)** showed more potent antibacterial activity (MIC=12.5 µg/mL, 25 µg/mL) nearly equivalent to that of ampicillin and gentamicin against all bacterial strains tested except for *P.aeruginosa.*

Also compound **(8)** has shown moderate activity towards the tested bacteria, except for *P.aeruginosa*. The MIC of the pyrimidine thione derivative **(5b)** was found to be about two fold less than that of chalcone **(1b)** and equal to the value of the chalcone **(1c)**. thus, the pyrimidine thione **(5b)** is as potent antibacterial agent as the chalcone **(1c)** and is more potent antibacterial than the chalcone **(1b)**. This might be due to the fact that chalcone **(1c)** contains the same 4,6-diaryl groups in the pyrimidine

thione moiety resulting in resemblance of the potential bactericidal effects. The potential bactericidal effects of **8** can be related to the ability of the pyrimidoquinazolone system to undergo a conjugated addition to a nucleiphilic group like a thiol group in an essential protein.

Antifungal studies: Antifungal activity was also done by disk diffusion method. For assaying antifungal activity *Aspergillus fumigatus, Syncephalastrum racemosum*, *Geotricum candidum* and *Candidum* and *Candida albicans* were recultured in DMSO by agar diffusion method[19,20]. Sabourauds agar media was prepared by dissolving peptone **(1g)**, D-glucose **(4g)** and agar **(2g)** in distilled water (100 ml) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. Twenty milliliter of agar media was poured into each Petridish, Excess of suspension was decanted and the plates were dried by placing in incubation at 37°C for 1h. Using an agar punch, wells were made and each was labeled. A control was also prepared in triplicate and maintained at 37°C for 3-4 days. The antifungal activity of each compound was compared with Amphotericine B as a standard drug.

Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 4. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and control was inoculated with approximately 1.6 x 10^4-6x10^4 c.f. u/ml. the cultures were incubated for 48h at 35°C. and the growth was monitored. The lowest concentration) (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (MIC).

Table 4. Antifungal activity of compounds (1b, 1c, 5b, 6-8): positive control (standard) Amphotericin B and negative control (DMSO) measured by the Halo Zone Test (unit, mm).

Comp. No.	Diameter of zone of inhibition (mm)			
	*AF	*SR	*GC	*CA
1b	13.8 ± 0.25	14.6± 0.58	17.5± 0.58	12.8± 0.25
1c	17.2± 0.44	15.1± 0.35	18.9± 0.58	14.6± 0.17
5b	16.8± 0.25	16.4± 0.29	19.3± 0.35	14.8± 0.58
6	22.8± 0.19	19.2± 0.44	25.9± 0.58	22.6± 0.17
7	NA	NA	NA	NA
8	19.8± 0.58	18.7± 0.58	20.8± 0.35	17.4± 0.0.35

* Where AF: *Aspergillus fumigatus,* SR: *Syncephalqstrum racemosum*; GC: *Geotricum candidum* ; CA: *Candida albicans.*

Table 5. MIC and MFC of compounds (1b,c,5b and 6-8), positive control is amphotericin B

Comp. No.	AF		SR		GC		CA	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
1b	100	100	100	100	50	50	100	100
1c	50	50	100	100	50	50	100	100
5b	100	100	100	100	50	50	100	100
6	6.25	12.5	6.25	12.5	12.5	25	25	25
7	>100	>100	>100	>100	>100	>100	>100	>100
8	25	50	50	50	25	50	100	100

AF: *Aspergillus fumigatus,* SR: *Syncephalastrum race-mosum*, GC: *Geotricum candidum,* CA: *Candida albicans.* MIC (µg/mL)= minimum inhibitory concentration, that is, the lowest concentration of the compound to inhibit the growth of fungus completely; MFC (µg/mL)=minimum fungicidal concentration, that is, the lowest concentration of the compound for killing the fungus completely

The antifungal screening data of compounds (1b,c; 5b, 6-8) revealed excellent to moderate activity. Compound **(6)** showed an excellent inhibitory activity against *A. fumigatus* (22.8 vs 23.7 standard); *S.racemosum* (19.2 vs 19.7 standard), *G. candium* (25.9 vs 28.7 standard) and *C.albicans* (22.6 vs 25.4 standard). Compound **(8)** showed moderate activity towards the four used fungal strains. The chalcone derivative **(1c)** and its derived pyrimidine thione **(5b)** were less active towards the four used fungal stains, whereas the chalcone derivative **1b** showed the least activity. Compound **(7)** was completely inactive towards any of the four used fungal strains, c.f Table 5.

Cytotoxic Activity

Cytotoxic activity of the synthsized derivatives **(1b, c, 2; 5a, b; 6-8 and 11)** was performed against three cancer cell lines namely, breast carcinoma cells (MCF-7), colon carcinoma cells (HCT) and cervical carcinoma cells (HeLa) using a modified method [21]. The results (Table 6) showed that the chalcone **(1b)**, the pyrimidine thiones

(5a,b) and the bis-disulphide pyrimine derivative **(7)** showed no cytotoxic activity against the HCT and MCF-7 cell lines with IC_{50} of => 50 µg/mL for all these derivatives. It is notable that **1b** has shown no cytotoxic activity against colon and breast carcinoma cells with the IC_{50} of => 50µg/mL for each kind. This is presumably due to the high lipophilicity of the α,β-unsaturated ketone chain which inhibits its absorption to the cancer cells. Compound **(8)** has shown a moderate cytotoxic activity against colon and breast cells (HCT and MCF-7) with the IC_{50} of 42.3, 38.5 µg/mL respectively. Compound **(1c)** exerted activity against cancer cells HCT with the IC_{50} of 30.3 µg/mL, this may be due to the hydrophobic nature of 8 and 1c. In addition, compounds (1c) and (11) displayed activity against MCF-7 and Hela cell lines (breast and cervical carcinoma cells) with the IC_{50} of 15.2µg/mL for each one of them. Further, compound **(6)** has shown cytotoxicity against HCT cell line with IC_{50} of 9.8 µg/mL and compound **(5b)** has shown cytotoxicity against MCF-7 cell line with IC_{50} of 8.3 µg/mL. Finally, compound **(2)** exerted a moderate cytotoxic activity against colon carcinoma cells with IC_{50} of 4.6 µg/mL with respect to the control, c.f. Table 6.

Table 6. Cytotoxic activity of some derivatives 1b, 1c, 2, 5a, 5b, 6,7,8,11 cell line[(a)] IC_{50} (µg/mL)[(b,c)].

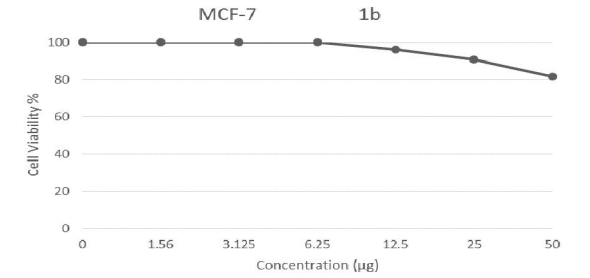
	1b	1c	2	5a	5b	6	7	8	11
HCT	>50	30.3	4.6	NT	>50	9.8	NT	42.3	NT
MCF-7	>50	15.2	NT	>50	8.3	NT	>50	38.5	
HeLa	NT	NT	NT	NT	NT	NT	NT	NT	15.2

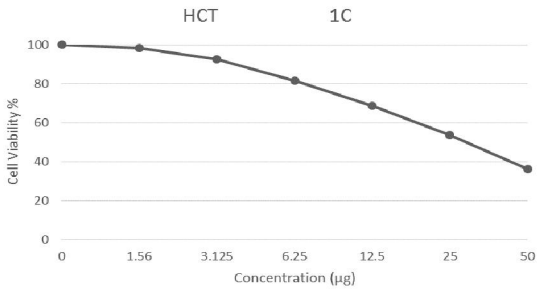
NT: indicates not tested

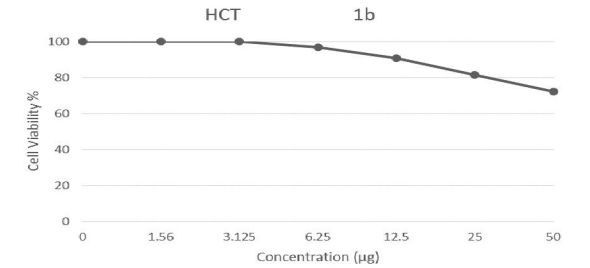
[a] cancer cell lines were human colon carcinoma (HCT), breast cancer cell line (MCF-7) and cervical adenocarcinoma cell line (He La).

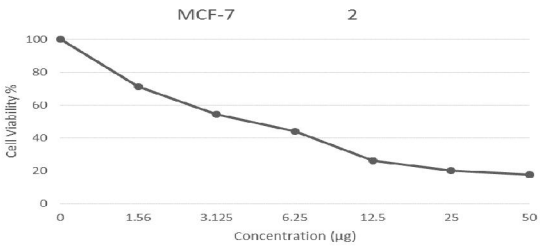
[b] when IC_{50} >50µg/mL denotes inactive compounds

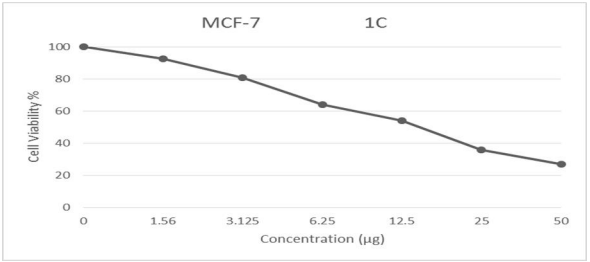
[c] The assays were performed intriplicate.

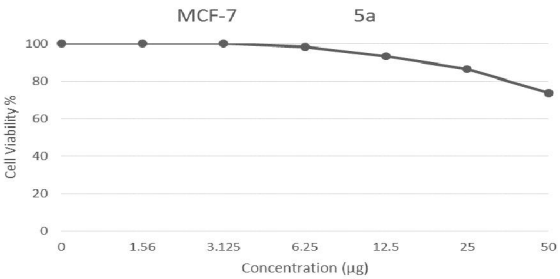


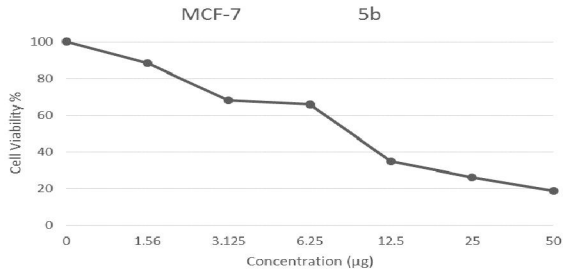


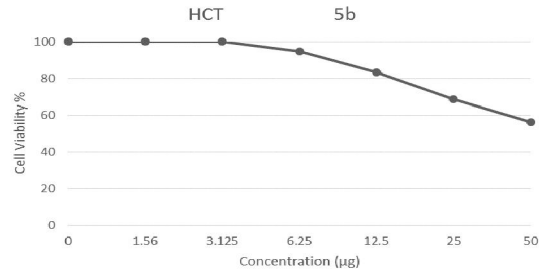


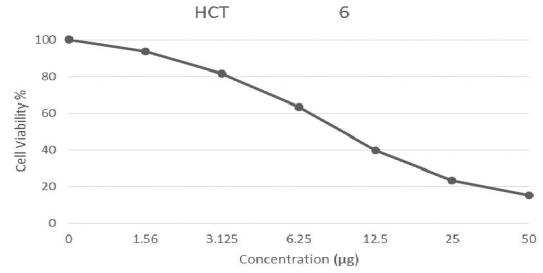


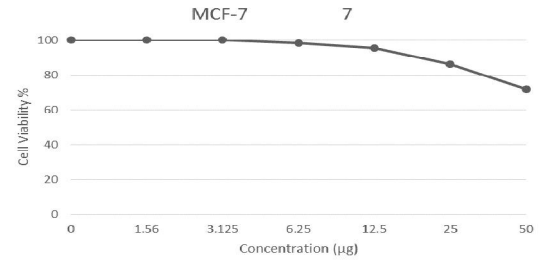


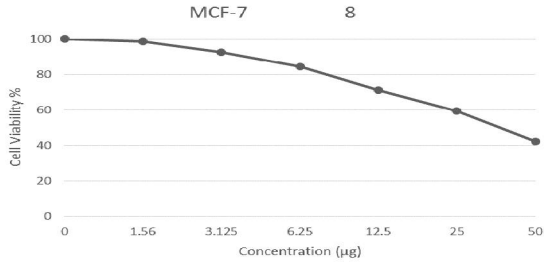


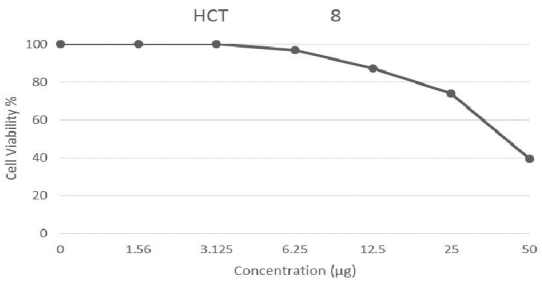


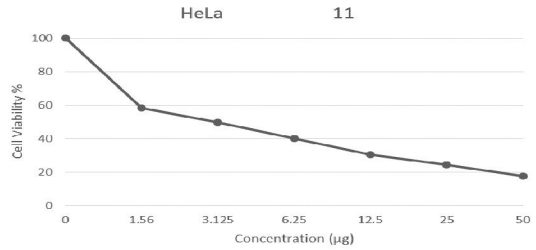










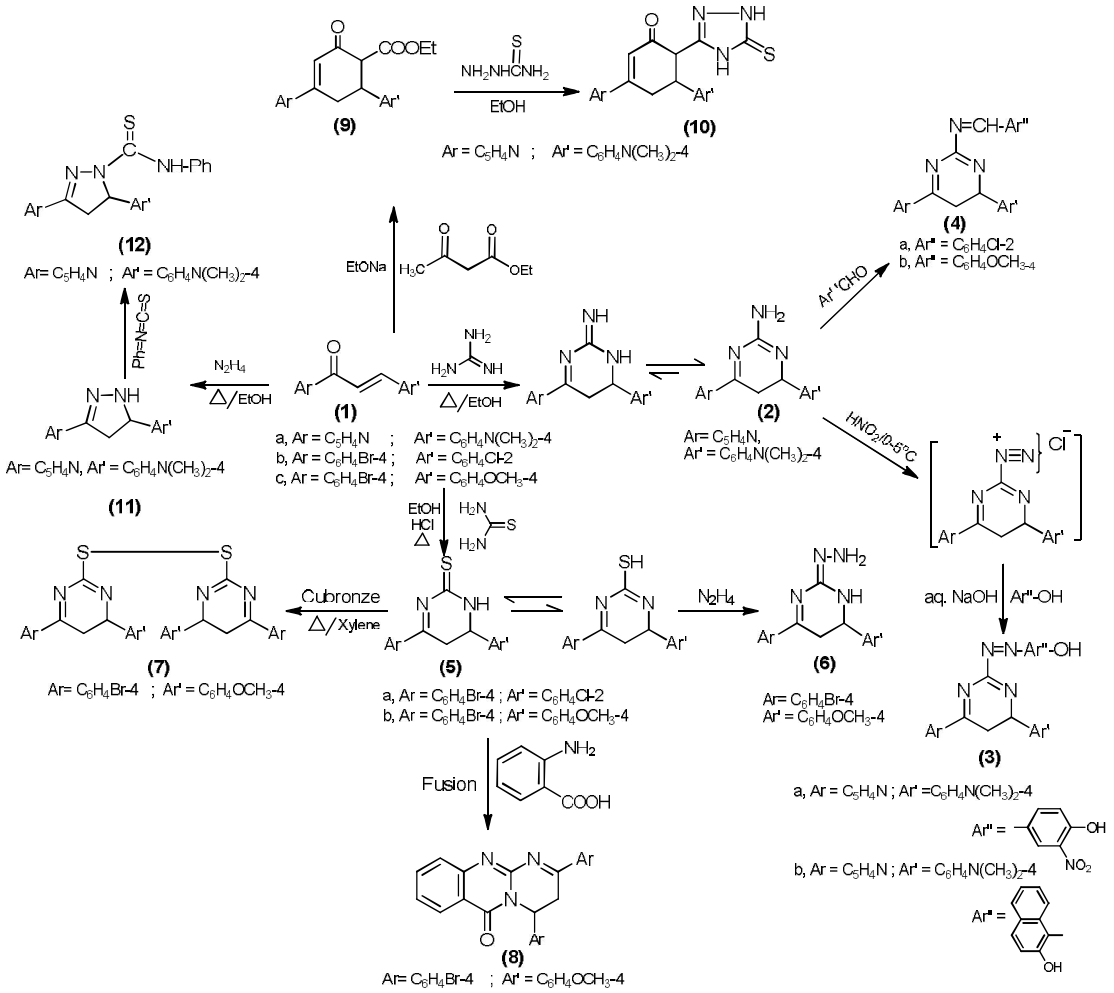


Conclusion

The chalcones **1a-c** reacted with nucleophilic reagents such as guanidine, thiourea to give the corresponding pyrimidine derivatives **2** and **5a,b**. the reaction of **1a** with ethyl acetoacetate gave the cyclohexenone derivative **9** and with hydrazine hydrate, it yielded the pyrazoline derivative **11**. The reaction of **5** with hydrazine hydrate gave 2-hydrazino pyrimidine **6** which exerted antigrowth activity against *A.fumigatus, S.racemosum G.candidum* and *C.alicans*. Significantly, compound **6** exerted complete inhibition against Gram-positive bacteria *S.pneumonae* and *B.subtilis* and Gram-negative bacteria *E.coli*. The pyrimidoquinzolone **8** displayed cytotoxic activity against two cell lines HCT and MCF-7 showing IC_{50} of 42.3 and 38.5 µg/mL. The chalcone **1c** exerted significant activity against HCT with IC_{50} of 30.3 µg/mL and against MCF-7 with IC_{50} of 15.2 µg/mL.

In conclusion, the study leads to the identification of novel antimicrobial **1c,6,8** and cytotoxic agents **1c, 5b**. Significantly, **6** exhibited both antibacterial and antifungal actions, whereas **2** was found to be the most potent cytotoxic compound against human colon carcinoma and against breast cancer cell line. The findings demonstrate a new potential for pyrimidine derivatives as lead compounds for further development as medicinal agents.

Reaction Scheme



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