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Synthesis of some novel pyrrolidine, thiomorpholine, pyrimidine and pyridine derivatives containing benzimidazole moiety of expected antiviral and antimicrobial activity

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ABSTRACT

A series of new substituted pyrrole, thiomorpholine-3, 5-dione, pyrimidine, thiazole and pyrazole derivatives were synthesized from N-(4-(1H-benzo[d] imidazol-2-yl) phenyl-2-chloroacetamide ***(2)*** *as starting material. Also, N (4-(1H-benzo[d]imidazol-2-yl) phenyl)-3-oxobutanamide* ***(17)*** *is widely used for production of pyridine and chromeno[3,4-c]pyridin-1-carboxamide derivatives. Most of the target compounds were evaluated for their antiviral and antimicrobial activities.*

Keywords: benzimidazole, pyrrolidine, pyridine, antiviral and antimicrobial activities.

INTRODUCTION

Literature survey showed that the compounds bearing benzimidazole moiety are reported to possess a number of interesting biological activities and the widespread importance of benzimidazole structure has extensive studies for practical synthetic method of heterocyclic compounds [1-5]. Benzimidazole derivatives play a vital role in biological activities such as anti-diabetic [6],antimicrobial [7,8], antifungal [9], antiviral [10, 11], antispasmodic [12], anticancer [13,14], anti-tumor [15],anti-hepatitis-C-virus [16], kinas inhibitor [17,18],analgesic [19], antipsychotic [20], antidepressant [21], anti-anxiety [22], anti-hypertensive [23], antiulcer [24] and anti-inflammatory [25]. Accordance to these observations, it has been considered to prepare new chemical entities that containing pyrimidine, pyrrole, thiazole, pyridine containing benzimidazole moieties as important potential pharmacologically important molecules.

MATERIALS AND METHODS

All melting points were determined in open capillaries and are uncorrected. Progress of the reaction was monitored by TLC plates; IR spectra were measured using (KBr) discs and a pye Unicom SP-1000 spectro-photometer. ^{1}H NMR spectra were measured on a Varian EM-390-200 MHz instrument in DMSO-d_6) as solvent using TMS as internal started and chemical shifts are expressed as δ ppm. Mass spectra were measured and a shimadzu G CMSQP-100 Ex mass spectrometer at 70 eV. Antiviral screening was carried out in Botany Department, Faculty of Science, Al-Azhar University and Antimicrobial activity screening was carried out in Biochemistry Department, Faculty of Agric., Al-Azhar University.

Synthesis of 1-(4-(1H-benzo[d]imidazol-2-yl) phenyl)-2-amino-5-oxo-4,5-dihydro-1H-pyrrol-3-carbonitrile (3), 1-(4-(1H-benzo[d] imidazol-2-yl) phenyl)-3-acetyl-1H- pyrrol-2,5-dione(8).

General procedure:

A mixture of **2** (0.01 mol) malononitrile and/or ethyl acetoacetate (0.1 mol) with anh. K_2CO_3 (0.5 gm) for each in dimethyl formamide (20 ml) was refluxed 6h, then the mixture was filtered when hot and allowed to cool. The solid product was collected and recrystallized from the proper solvent to give **3** and **8** respectively.

Compound (3) This compound was recrystallized from dimethyl formamide as deep violet crystals in 73% yield, m.p. 255-257°C. IR (KBr, cm^{-1}): 1663 (CO), 2200 (C≡N), 3133 & 3435 (NH_2/NH). ^{1}H NMR (DMSO-d_6): δ 3.13 (br, 2H, NH_2), 3.99 (s, 2H, CH_2), 6.74 (d, 2H,*J*= 7.02Hz , Ar–H), 7.13-7.95 (m, 6H, Ar–H), 8.74 (s, 1H, NH, exchanges with D_2O) MS: m/z: 315 (M^+, 8.1), 285 (25), 185 (33.7), 67 (49.1) and 58 (100). Anal. Calcd. for $C_{18}H_{13}N_5O$ (315.34): C, 68.56; H, 4.16; 22.21. Found: C, 68.51; H, 4.11; N, 22.19.

Compound (8).this compound was recrystallized from dimethyl formamide as brown crystals in 65% yield, m.p. 275-278°C. IR (KBr, cm^{-1}): 1661(3CO), 3212 (NH). ^{1}H NMR (DMSO-d_6): δ 2.47 (s, 3H, $COCH_3$), 5.81 (s, 1H, CH-pyrrole) , 7.47-8.22 (m, 8H,Ar–H) , 10.16 (s1H, NH, D2Oexchangeable). Anal. Calcd.for $C_{19}H_{13}N_3O_3$ (331.11): C, 68.88; H, 3.95; N, 12.68. Found: C, 68.68; H, 3.84; N. 12.46.

1-(4-(1H-benzo[d]imidazol-2-yl) phenyl)-2, 5-dioxopyrrolidin-3-carbonitrile (5).

Equimolar amounts of **2** (0.01 mol) and ethyl cyanoacetate (0.01 mol) with piperidine (0.5 mol) in ethanolic dimethyl formamide mixture were refluxed for 6h. The solid product was collected and recrystallized from dimethyl formamide as brown crystals in 81% yield, m.p. > 360°C. IR (KBr, cm^{-1}) 1658 (2CO), 2201(CN), 3441 (NH). ^{1}HNMR (DMSO-d_6): δ 2.81 (q, 1H, CH), 4.04 (q, 2H, CH_2), 6.74 (d, 2H, *J=7.11Hz*,Ar–H), 7.01-8.22 (m, 7H , Ar–H, + NH), Anal. Calcd. for $C_{20}H_{18}N_4O_3$ (362.39): C, 66.29; H, 5.01; N, 15.46: Found: C, 66.11; H, 5.01; N, 15.24.

4-(4-(1H-benzo[d] imidazol-2-yl) phenyl) thiomorpholin-3, 5-dione (9)

A mixture of **2** (0.01 mol) and mercapto acetic acid (0.01 mol) in pyridine (30 mol) was refluxed for 8h. The reaction mixture was cooled and poured into ice and dil.HCl, then the solid obtained was filtered and crystallized from chloroform to give **9** as brownish red crystals in 66% yield, m.p.>360°C. IR (KBr, cm^{-1}): 2923 (2=CH), 3437 [NH/2OH]. ^{1}H NMR (DMSO-d_6): δ5.72 (s, 2H, 2=CH), 7.21(d, 2H , J=7.01Hz,Ar–H) 7.58-8.68 (m, 6H, Ar–H), 9.07 (s, 1H, NH D_2O exchangeable), 11.23 (s, 2H, 2OH, D_2O exchangeable). MS: m/z: 323 (M^+, 17.22), 209 (68), 118 (31), 91(39), 79(100). Anal. Calcd. for $C_{17}H_{13}N_3O_2$ S (323.37): C, 63.14; H, 4.05; N, 12.99; S,9.91. Found: C, 63.11; H, 4.00; N, 12.81; S, 9.90.

N-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)amino) acetamide (10).

A mixture of 2 (0.01 mol) and antipyrene (0.01 mol) with anhydrous potassium carbonate (0.5 gm) in ethanol (25 ml) was refluxed for 4h. The reaction mixture was filtered on hot and the resulting solid was crystallized from ethyl acetate as deep orange crystals, m.p.> 350^0C. IR (KBr, cm–1): 1664 (2CO), 3430 (br, 3NH) MS: m/z = 452 (M+, 6.18), 409 (10) 369 (17.12), 57 (98.1), 43(100). Anal Calcd. for $C_{26}H_{24}O_2N_6$ (452.51): C, 69.01; H, 5.35; N, 18.57. Found: C, 69.00; H, 5.17; N,18.38.

4-(furan-2-yl)-5,6,7,8-tetrahydroquinazoline-2-thiol (11).

A mixture of 2-(furan-2-ylmethylene) cyclohexan-1-one (0.01 mol), thiourea (0.012 mol) and potassium hydroxide (0.5g) in ethanol (30 mL) was heated under reflux for 3h, the reaction mixture was cooled then acidified with dil. HCl. The solid product was collected and recrystallized from ethanol to give 10 as yellow powder; m.p. 139–143^0C.IR (KBr, cm–1):1211(CS), 2841 &2911 (CH_2-Aliph.)),3231(NH), 1H NMR (DMSO–d6): δ1.23-2.12 (m, 8H, Aliph.), 6.31 (d, 1H, *J=2.01Hz*, C–3 furan), 6.91 (m, 1H, C–4 furan), 7.71 (d,1H,*J=2.21Hz*, C–5furan)), 1013 (s, 1H, NH, exchanges with D_2O). Anal Calcd. For $C_{12}H_{12}N_2OS$ (232):C, 62.05; H, 5.21; N, 12.06. Found: C, 69.10; H, 5.17; N,12.38.

N-(4-(1H-benzo[d]imidazole-2-yl)phenyl)-2-(4-(furan-2-yl)-5,6,7,8-tetrahydroquinazolin-2-yl)thioacetamide (12).

Equimolar amounts of **2** (0.01 mol) and **11** (0.01 mol) with anhydrous potassium carbonate (0.5 gm) in dry acetone (30 ml) were refluxed for 8h. The mixture was filtered on hot and allowed to cool. The solid product was

crystallized from acetone as violet crystals in 55% yield, m.p. 248-250°C. IR (KBr, cm^{-1}): 1661 (CO), 2855&2921 (CH_2-Aliph.),3426 (2NH), ^1H NMR (DMSO–d_6): δ 1.23-2.12 (m, 8H, Aliph.), 4.12 (s, 2H, CH_2), 6.03 (d, 1H, J=2.01Hz, C_{-3} furan), 6.31 (q, 1H, C_4 furan), 6.7 (d, 2H, J=7.12 Hz, Ar–H), 7.24-8.1 (m 8H, Ar–H + 1H, C_5 furan + NH), 10.55 (s, 1H, NH, exchanges with D_2O). MS: m/z = 481 (M^+, 7.11), 402 (11.2), 325 (32.7) 106 (66) with a base peak 43. Anal. Calcd.for $C_{27}H_{23}N_5O_2S$ (481.57): C, 67.34; H, 4.81; N, 14.54; S, 6.66. Found: C, 67.25; H, 4.70; N, 14.33; S, 6.61.

N-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-2-((4-(furan-2-yl)-5,6,7,8-tetra-hydroquinazolin-2-yl)sulfonyl) acetamide (13).

A mixture of **12** (0.01 mol) and hydrogen peroxide (2 ml) in acetic acid (15 ml) was refluxed for 3h., the solid product was collected and crystallized from dimethyl formamide as brown red crystals in 71% yield, m.p.> 360°C. IR (KBr, cm^{-1}): 1138 & 1389 (SO_2), 1663 (CO), 2911 & 2833 (CH_2Aliph.), 3461 (br, 2NH). MS: m/z = 513 (M^+, 11.3), 446 (6.59), 376 (16.1), 200 (5.11), 106 (69), 41(100). Anal.Calcd.for $C_{27}H_{23}N_5O_4S$ (513.57): C, 63.15; H, 4.51; N, 13.64; S,6.24.Found: C, 63.11; H, 4.41; N, 13.59; S,6.22.

N-(4-(1H-benzo[d] imidazol-2-yl) phenyl-2-thiocyanato acetamide (15).

A mixture of **2** (0.01 mol) and potassium thiocyanate (0.01 mol) dissolved in dimethyl formamide (30 ml) was heated and refluxed for 6h. the reaction mixture then cooled and poured into crsushed ice and acidified with dil. HCl. The solid product was collected and crystallized from chloroform as blue crystals in 56% yield, m.p. 290°C. IR (KBr, cm^{-1}): 1671 (CO), 2190 (C≡N), 3393 & 3427 (2NH). ^1H NMR (DMSO-d_6): δ 3.38 (s, 2H, CH_2), 6.69-8.71 (m, 9H, Ar–H+NH), 9.89 (s, 1H, NH, D_2O exchangeable).Anal Calcd.for $C_{16}H_{12}N_4SO$ (308.36): C, 62.32 ; H, 3.92 ; N, 18.17 ; S, 10.40. Found: C, 62.22 ; H, 3.88 ; N, 18.15 ; S, 10.27.

N^4 (4-(1H-benzo[d] imidazol-2-yl) phenyl)-1, 3-thiazol- 2,4-diamine (16)

A mixture of 2 (0.008 mol) and thiourea (0.02 mol) in ethanol/dimethyl formamide mixture was refluxed 2h. the solid product was collected and crystallized from chloroform as brownish red crystals in 87% yield, m.p. 310^0C IR (KBr, cm^{-1}) 3312 & 3429 (NH_2/2NH). MS: m/z = 322 (M+, 23), 307 (3.2), 244(5.5), 197 (7.01), 69.1 (99.8), 43 (100). Anal.Calcd.for C17H14N5S (322.31): C, 63.73; H, 4.40; N, 21.86; S, 10.01. Found: C, 63.64; H, 4.35; N, 21.81; S, 10.02.

N-(4-(1H-benzo[d] imidazol-2-yl) phenyl)-3-oxo butanamide (17).

A mixture of **1** (0.01) mol and ethyl acetoacetate (0.0l mol) in presence of piperidine (2 ml) in dimethyl formamide (30 ml) was refluxed 8h. The reaction mixture was poured into crushed ice and acidified with dil. HCl. The solid was filtered off and recrystallized from acetone as deep violet crystals in 86% yield, m.p. 296-300°C. IR (KBr, cm^{-1}): 1663 & 1681 (2CO), 3334 & 3427 (2NH). ^1H NMR (DMSO-d_6): δ: 2.49 (s, 3H, $COCH_3$), 4.07 (s, 2H, CH_2) 6.64-7.84 (m, 8H, Ar–H), 8.11 (s, 1H, NH, D_2O exchangeable) and 10.54 (s, 1H, NH, D_2O exchangeable). Anal.Calcd.for $C_{17}H_{15}N_3O_2$ (293.32) C, 69.61; H, 5.15; N, 14.33. Found : C, 69.58; H, 5.11; N, 14.17.

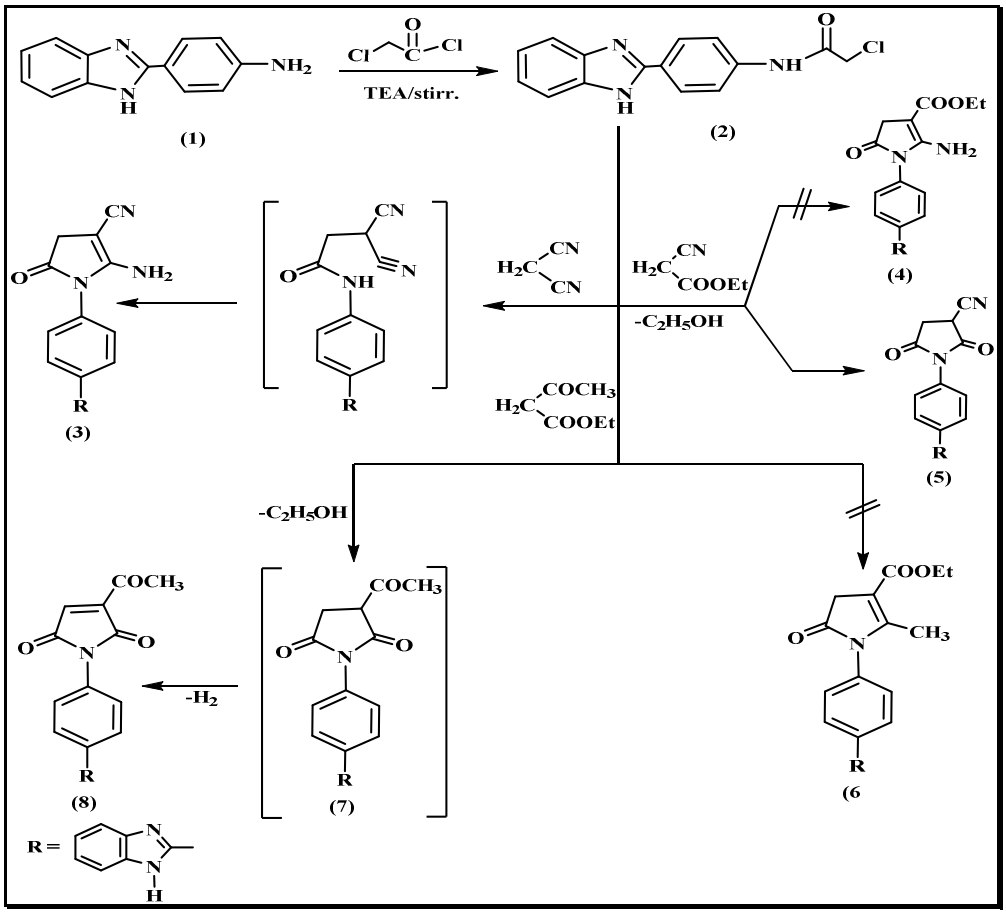
6-(4-(1H-benzo [d] midazol-2-yl) phenyl amino)-2-amino-4-methyl pyridin-3-carbonitrile (18).

Equiomolar amounts of **17** (0.01 mol), malononitrile(0.01 mol) in presence of ammonium acetate (2gm) in ethanolic dimethyl formamide mixture (20 ml) were refluxed 10h. the solvent was evaporated and the solid product was crystallized from acetone as brown crystals in 70% yield, m.p. > 360°C. IR (KBr, cm^{-1}): 1628 (C=N), 2198 (C≡N), 3436 (br, NH_2/2NH). ^1H NMR (DMSO-d_6): δ: 2.22 (s, 3H, CH_3), 4.51 (br, 2H, NH_2), 6.46 (s, 1H, CH pyridine), 7.18-8.44 (m, 9H, Ar–H +NH), 10.21 (s, 1H, NH, exchanges with D_2O). Anal Calcd. for $C_{20}H_{16}N_5$ (340.39): C, 70.57; H, 4.74; N, 24.69. Found: C, 70.32; H, 4.71 ; N, 24.66.

6-((4-(1H-benzo[d]imidazol-2-yl) phenyl amino)-2-hydroxy-4-methyl pyridin-3-carbonitrile (19).

A mixture of **17** (0.01 mol) and cyano acetamide (0.01 mol) in presence of ammonium acetate was fused on a hot plate.The solid product was collected and recrystallized from acetone as deep violet crystals in 67% yield, m.p. 191-193°C. IR (KBr, cm^{-1}): 1623 (C=N), 2221,(CN), 3313 &3437 (OH/2NH). ^{1}HNMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 6.35 (s, 1H, CH pyridine), 6.66(d, 2H, J=8.1 Hz, Ar–H)7.17-8.5 (m, 7H, Ar–H + NH), 8.88 (s, 1H, NH, exchanges with D_2O), 10.48 (s, 1H, OH, D_2O exchangeable). Anal. Calcd.for $C_{20}H_{15}N_5O$ (341.37): C, 70.37; H, 4.43; N, 20.52. Found : C, 70.33; H, 4.26; N, 20.41 .

N-(4-(1H-benzo[d] imidazol-2-yl)phenyl)-4-amino-2-methyl-5-oxo-5H-chromeno [3,4-c]-1-carboxamide (22). A mixture of **17** (0.0 mol) and 2-oxochromen-3-carboximidamide (0.01mol) in ethanol (30 ml) was refluxed 8h, and then allowed to cool. The solid product was crystallized from acetone as brown crystals in 66% yield, m.p. 280-282°C. IR (KBr,cm^{-1}): 1633(CONH), 1704(CO) of α–pyrone, 3233-3430 (NH_2/ 2NH), ^{1}H NMR (DMSO-d_6): δ 2.2 (s, 3H, CH_3), 6.67 (d, 2H,Ar–H), 7.15-8.11 (m, 10H, Ar–H), 9.13 (s, 1H, NH, D_2O exchangeable). MS: m/z: 461 (M^+, 9.3%), 377 (2), 281 (88.1), 97(27), 57 (100). Anal.Calcd.for$C_{27}H_{19}N_5O_3$ (461.47): C, 70.27; H, 4.15; N, 15.18. Found: C, 70.14; H, 4.01; N, 15.03.



Scheme (1) synthesis of pyrrolidine derivatives

RESULTS AND DISCUSSION

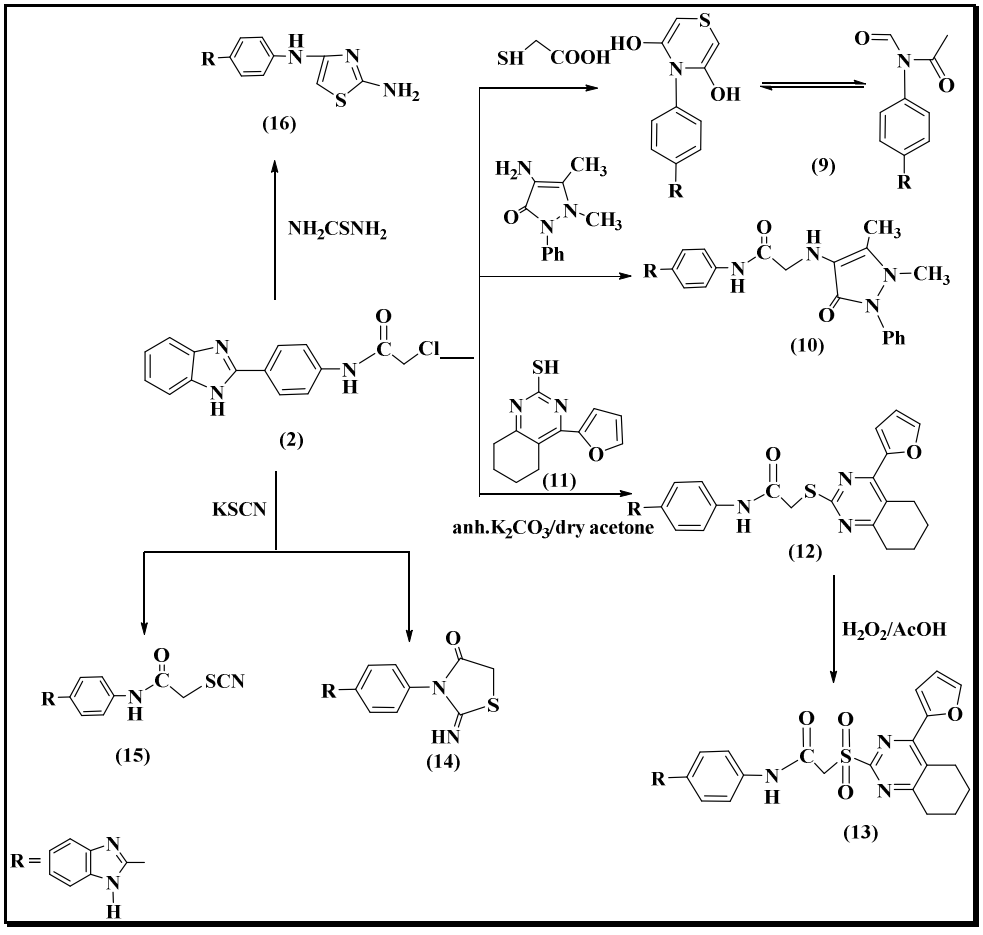
N-(4-(1H-benzo[d]imidazol-2-yl) phenyl-2-chloroacetamide **(2)** was prepared from the interaction of 4-(1H-benzimidazol-2-yl)benzenamine **(1)** [26] with chloroacetyle chloride in presence of triethyl amine according to the reported method [27]. Chloroacetamide derivative **(2)** is considered to be useful starting material for the synthesis of a novel heterocyclic compounds and the reactivity of **2** towards active methylene compounds were investigated. Thus, condensation of **2** with malononitrile gave 5-oxopyrroliden-3-carbonitrile derivative **(3) (**scheme 1) the formation of **3** is assumed to proceed via alkylation of malononitrile followed by intramolecular cyclization to furnish pyrrole **(3)**.

The structure of **3** was confirmed by elemental analysis and spectral data. IR spectrum of **3** showed bands at 1663 cm^{-1} for (>CO) group, 2200 for (C≡N) and 3133 & 3435 for (NH_2/NH) groups. Its ^{1}H NMR spectrum showed a signal at δ 3.13 ppm (br, 2H, NH_2), 3.99 (s, 2H, CH_2), 6.74 (d, 2H, Ar–H), 7.13-7.95 (m, 6H, Ar–H) and 8.74 (s, 1H, NH, D_2O exchangeable). Also, cyclization took place by treatment of compound **2** with ethylcyanoacetate hoping to afford ethyl-4-oxo pyrroliden-3-carboxylate **(4)** but 2,5-dioxopyrrol-3-carbonitrile **(5)** was obtained. The

formation of compound **5** produced through initial alkylation of ethyl cyanoacetate followed by intramolecular cyclization to form **5 (**scheme 1**).** The structures of **5** has been assigned as a reaction product on the basis of analytical and spectral data. IR spectrum of **5** revealed band sat 1658 cm^{-1}, 2201 and 3441 for 2CO, CN and NH.

In addition, cyclization of compound **2** with mercapto acetic acid gave thiomorpholin-2, 5-dione or the possible isomer **(9)** (scheme 2).The IR spectrum of **9** showed a band at 2923 (2=CH) and 3437 [NH/2OH] groups, ^{1}H NMR spectrum of compound **9** afforded signals at δ 5.72 (s, 2H, 2=CH), 7.21(d, 2H, Ar–H),7.58-8.68(m, 6H, Ar-H), 9.07 (s, 1H, NH, D_2O exchangeable) and 11.23 (s, 2H, 2OH, D_2O exchangeable). On the other hand, compound **2** is considered as key intermediate for synthesis of some acetamido derivatives. Thus, treatment of compound **2** with antipyrene gave N-(4-(1H-benzo[d] imidazol-2-yl) phenyl)-2-(2,3-dimethyl-5-oxo-1-phenyl pyrazol-1-yl) amino acetamide (**10**) (scheme 2) which confirmed by spectral data and its IR spectrum showed a broad band at 1664 cm^{-1} for (2CO) and 3430 (br, 3NH) groups. The mass spectrum of compound **10** showed a molecular ion peak at m/z 452 (6.18) which is in agreement with its molecular formula $C_{26}H_{24}O_2N_6$.

Moreover, condensation of **2** with 5, 6,7,8-tetrahydroquinazoline-2-thiol derivative **(11)** in presence of anhydrous potassium carbonate gave 4-(4-(1H-benzo[d]imidazol-2-yl) phenyl) -2-((4-(furan-2-yl)-5,6,7, 8 tetrahydro quinazolin-2-yl) thioacetamide **(12)** which oxidized by hydrogen peroxide to afford sulfonyl acetamide derivative **(13)** (scheme 2). Compounds **12** and **13** were established by elemental analysis and spectral data. ^{1}H NMR spectrum of **12** showed signals at δ 1.23-2.12 ppm (m, 8H, aliph.), 4.12 (s, 2H, CH_2), 6.03 (d, 1H, C_{-3} furan), 6.31 (q, 1H, C_{-4} furan) 6.7 (d, 2H, Ar–H), 7.24-8.1 (m, 8H, 6Ar–H + 1H, C_{-5} furan + NH) and 10.55 (s, 1H, NH, D_2O exchangeable). The mass spectrum of compound **12** showed a molecular ion peak at m/z 481 (7.11) while, the mass spectrum of **13** exhibited a molecular ion peak at m/z 513 (11.3).



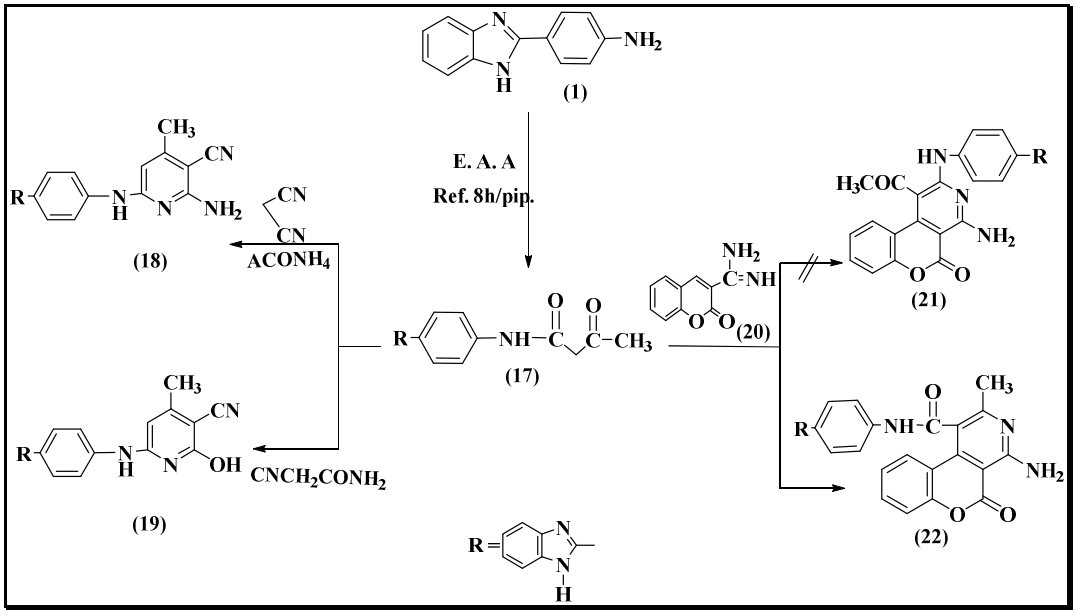
Scheme (2) synthesis of acetamido derivatives

In addition, interaction of **2** with potassium thiocyanate hoping to afford 2-iminothiazolidinone derivative **(14)** but thiocyanate acetamide derivative **(15)** (scheme 2) was obtained .The analytical and spectral data are in good agreement with structure **(15)**. IR spectrum of **15** exhibited bands, at 1671, 2190, 3393 , 3427cm^{-1} due to (CO) , (CN), and (2NH) groups respectively.

Finally, interaction of compound **2** with thiourea produced 2-amino thiazole derivative **(16)** (scheme 2). The IR spectrum of **16** exhibited the disappearance of carbonyl group found in the parent and exhibited bands at 33128 &3429 cm^{-1} for (NH_2/2NH), the mass spectrum of **16** showed a molecular ion peak at m/z 322 with a base peak at 43.

On the other hand, the compound of N-(4-1H-benzo[d]imidazol-2-yl)phenyl)-3-oxobutanamide **(17)** was obtained by treatment of **1** with ethylacetoacetate in presence of piperidine in dimethyl formamide. Compound **17** was confirmed by elemental analysis and spectral data. IR spectrum of **17** showed bands at 1663 & 1681 cm^{-1} for (2CO) and 3334 & 3427 for (2NH) groups. Also, its^{1}H NMR spectrum supported its structure , as it revealed signals at δ 2.49 ppm for ($COCH_3$) and 4.07 for (CH_2),6.64 (d,2H,Ar–H),7.08-7.84(m,7H, Ar–H +NH) and10.54 (s,1H , NH) and this was analogy according to previous work [28]. The reactivity of **17** towards some active methylene compounds was investigated. Thus, treatment of **17** with malononitrile or cyanoacetamide gave the corresponding 2-amino-4-methyl pyridin-3-carbonitrile derivative **(18)** and 3-cyano-4-methyl pyridin-2-o1 derivative **(19)** respectively (scheme 3).

The structures of **18** and **19** were supported with elemental analysis and spectral data. IR spectrum of **18** indicated the disappearance of carbonyl groups found in the parent and exhibited bands at 1628 cm^{-1} for (C=N), 2198 for (C≡N) and abroad band at 3436 for (NH_2/ 2NH) groups and^{1}H NMR spectrum showed signals at δ2.22 ppm (s, 3H, CH_3), 4.51 (br, 2H, NH_2), 6.46 (s, 1H, CH pyridine) 7.18-8.44 (m, 9H, Ar–H, +NH) and 10.21(s,1H, NH). IR spectrum of compound **19** lacked an absorption band due to a carbonyl functional group and reveald absorption bands at 1623 cm^{-1} for (C=N), 2221 for (C≡N) and bands at 3313 &3437for (2NH/ OH) groups,^{1}H NMR spectrum revealed signals at δ 2.3 ppm (s, 3H, CH_3) and 6.35 (s, H, CH pyridine).



Scheme (3)synthesis of pyridine derivatives

In addition, treatment of **17** with 2-oxochromen-3 carboximidamide **(20)** [29] hoping to obtain 1- acetyl-4-amino-5H-chromeno[3,4-c]pyridin-5-one**(21)** but N-(4-(1H-benzo[d] imidazol-2-yl)phenyl)-4-amino-2-methyl-5-oxo-5H-chromeno [3,4-c]-1-carboxamide **(22)** was obtained and **21** was eliminated scheme (3).

The product of this reaction was identified on the basis of its spectral data, IR spectrum of **22** exhibited a band at 1633 cm^{-1} for (CONH), a strong band at1704 for (CO) of α-pyrone and 3233&3430 (NH_2/ 2NH) groups .Also, its ^{1}HNMR spectrum showed signals at δ 2.2 ppm (s, 3H, CH_3), 6.67(d, 2H,ArH), 7.15-8.11(m, 12H, Ar–H) , 8.95, 9.13(2s, 2H , 2NH).

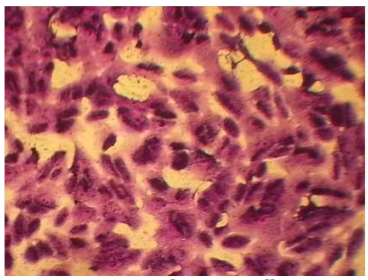
BIOLOGICAL EVALUATION

Antiviral activity

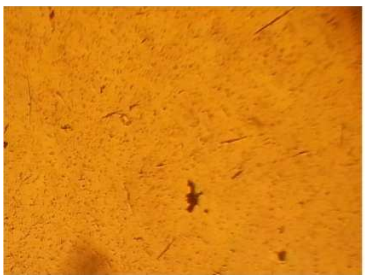
Compounds **5**, **15,** and **19** were studied for their antiviral activity against HSV-I virus and the results in table (1) revealed that compound **15** was found to highly potent to presence of isothiocyanate group. Compound **5** has moderate activity in decreasing order while, compound **19** showed the lowest activity.

Table (1): Antiviral effect against HSV-I virus

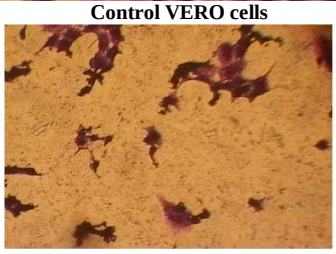
Test	O.D			MeanO.D	viability	toxicity	Viral activity	Antiviral activity
Control (VERO cell line)	0.246	0.248	0.241	0.245	100	0	------	-------
Virus control	0.16	0.152	0.15	0.154	62.85714	37.14285714	100	0
5	0.199	0.201	0.208	0.202667	82.72109	17.27891	46.52	53.48
15	0.219	0.214	0.209	0.214	87.34694	12.65306	34.06	65.94
19	0.177	0.189	0.182	0.182667	74.55782	25.44217	68.49	31.51



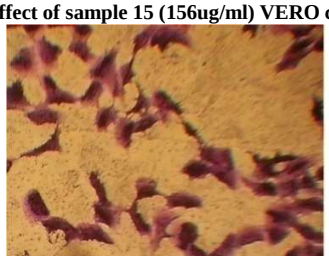
Control VERO cells



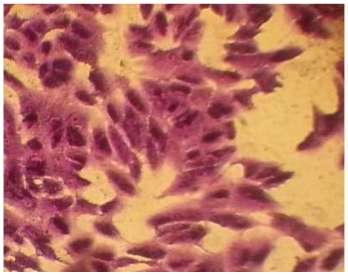
Effect of sample 15 (156ug/ml) VERO cells



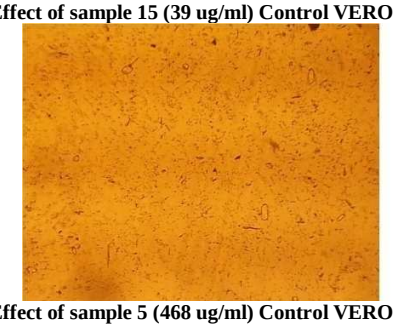
Effect of sample 15 (78 ug/ml) Control VERO cells



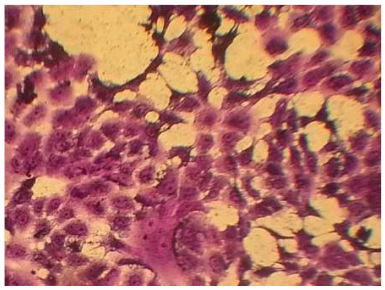
Effect of sample 15 (39 ug/ml) Control VERO cells



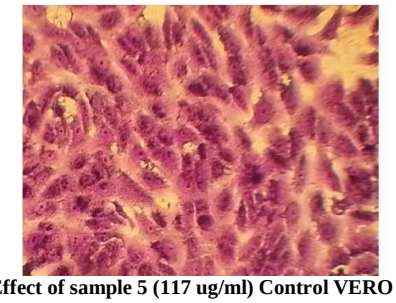
Effect of sample 15 (19.5 ug/ml) Control VERO cells



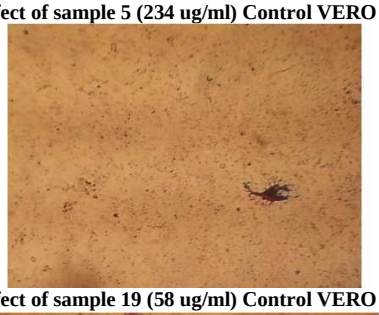
Effect of sample 5 (468 ug/ml) Control VERO cells



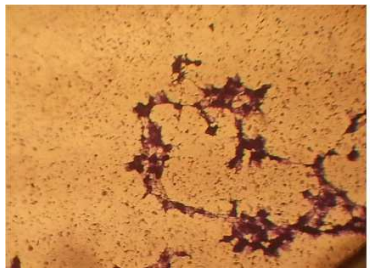
Effect of sample 5 (234 ug/ml) Control VERO cells



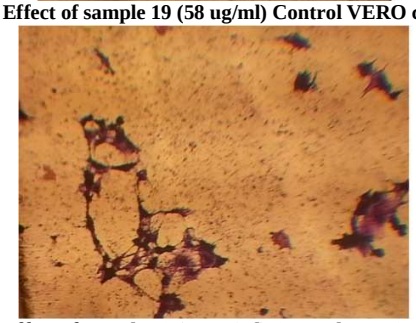
Effect of sample 5 (117 ug/ml) Control VERO cells



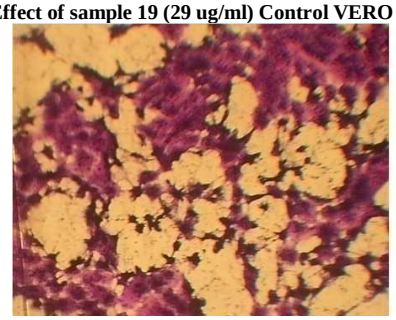
Effect of sample 19 (58 ug/ml) Control VERO cells



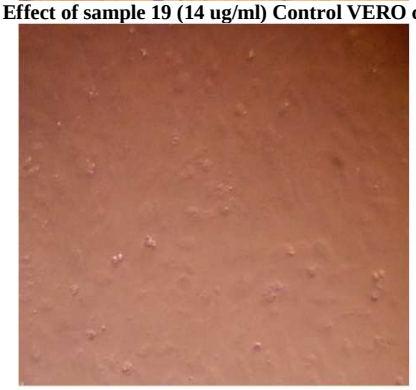
Effect of sample 19 (29 ug/ml) Control VERO cells



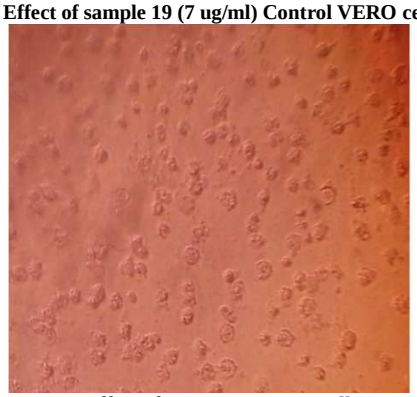
Effect of sample 19 (14 ug/ml) Control VERO cells



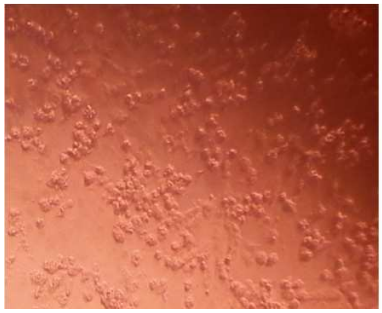
Effect of sample 19 (7 ug/ml) Control VERO cells



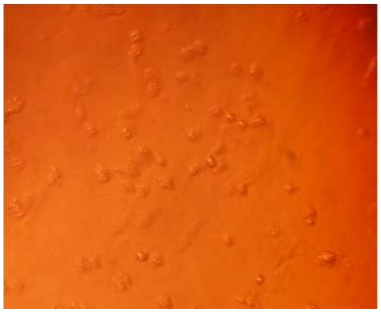
Control VERO cells



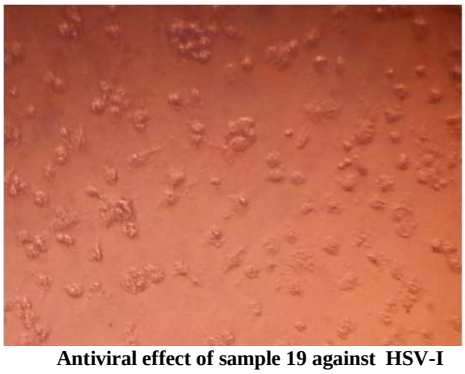
Effect of HSV-I on VERO cells



Antiviral effect of sample 15 against HSV-I



Antiviral effect of sample 5 against HSV-I



Antiviral effect of sample 19 against HSV-I

Antimicrobial activity

The antimicrobial activity data of some new synthesized compound were listed in table (2) which indicated that most of the synthesized compounds showed moderate to high activities. Compound **19** was effective against all tested microorganisms but not against *Aspergillus fumigatus.* Also it is worth mention that compounds **8**, **12**, **18** and **22** were no effective against all tested microorganisms.

Table (2): Antimicrobial activity of some new synthesized compounds

Organism	Mean' of zone diameter, nearest whole mm.											
	Gram-positive bacteria				Gram – negative bacteria				Yeast / Fungi**			
	Staphylococcus aureus (ATCC 25923)		*Bacillus subtilis* (ATCC 6635)		*Salmonella Typhimurium* (ATCC 14028)		*Escherichia coli* (ATCC 25922)		*Candida albicans* (ATCC 10231)	*Aspergillus fumigatus*		
Concentration / Sample	1 mg/m1	0.5 mg/ml	1 mg/m1	0.5 mg/ml	1 mg/m1	0.5 mg/ml	1 mg/m1	0.5 mg/ml	1 mg/m1	0.5 mg/ml	1 mg/m1	0.5 mg/ml
2	-	-	23 I	21H	-	-	-	-	19I	16I	-	-
3	-	-	-	-	-	-	-	-	23I	19H	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	20 I	18H	-	-	-	-	24H	19H	-	-
9	-	-	23 I	19H	-	-	-	-	24H	23H	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	21 I	18I	-	-
15	-	-	21 I	18H	-	-	-	-	24 H	20H	-	-
17	-	-	22 I	18H	-	-	-	-	16I	14I	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-
19	15I	14I	25 H	20H	12I	11 I	16I	11I	29H	25H	-	-
22	-	-	-	-	-	-	-	-	-	-	-	-
Control #	35	26	35	25	36	28	38	27	35	28	37	26

** Activity in comparison to references drug against tested organisms.*

*** identified on the basis of routine cultural, morphological and microscopical characteristic*

I: Intermediate activity = Mean of zone diameter < 2/3 of mean zone diameter of control.

H: High activity = Mean of zone diameter > 2/3 of mean zone diameter of control.

#: Chloramphencol in the case of Gram-positive bacteria, Cephalothin in the case of Gram-negative bacteria and cycloheximide in the case of fungi.

Antiviral activity:

Healthy cells were propagated as follows

1. The media overlaying cell monolayer was poured off.
2. 2- Cells can be released from tissue culture flask by treatment with about 5 ml pre- warmed trypsin-EDTA solution. (Trypsin cleaves cell surface proteins that the cells used to adhere to the flask. EDTA chelates metal ions that are involved in cell adherence], the flask was rocked so that trypsin completely cover the cell monolayer.
3. The trypsin was aspirated with a pipette, then 2 ml of trypsin were dispensed, the bottle rocked and was incubated at 37°C.
4. Cells were examined from time to time to avoid trypsin over action. The bottle was struck with hand to completely dislodge the cells from the bottle surface

5. Cells were suspended in about 8 ml of growth media. Use 10 ml pipette to disperse cell aggregates by sucking up and expel the cells about 4 times, expel the media with the tip of pipette pressed against the bottom of the bottle to ensure that no clumps of cells are present.
6. Cells were counted using haemocytometer and using trypan blue vital stain.
7. About 10 ml of $2x10^5$ VERO cell suspension, were transferred to 50 cm^3 TC bottle **(Falcon)** tightly closed then wasincubated at 37°C. Cells were sub-cultured once weekly.
8. For seeding 96 well plate, 0.1 ml (2×10^5 cells) was transferred to each flat bottomed well and incubated at 37°C for 24 – 48 hours to develop a complete monolayer sheet.

Determination of sample cytotoxicity on VERO cell (MTT protocol)
1. The dried extract was dissolved in 1 ml DMSO.
2. Growth medium was decanted from 96 well micro titer plates after confluent sheet of VERO cell was formed, cell monolayer was washed twice with wash media, then about 1 ml of wash media was added and the plates were incubated at room temperature for 5-10 minutes.
3. Double -fold dilutions of different extracts were made in MEM ,
4. 0.1 ml of each dilution was tested different wells leaving 6 wells as control, receiving only maintenance medium.
5. Plate was incubated at 37°C and examined frequently for up to 3 days. Cells were checked for any physical signs of toxicity, e.g. partial or complete loss of the monolayer, rounding, shrinkage, or cell granulation.
6. The maximum non-toxic concentration [MNTC] of each extract was determined and was used for further biological studies.

Antiviral assay
Procedure:
1. Plate 10,000 cells in 200ul media per well in a 96 well plate. Leave 8 wells empty for blank controls.
2. Incubate (37C, 5% CO_2) overnight to allow the cells to attach to the wells.
3. Incubate equal volume (1:1 v/v) of non lethal dilution of tested sample and the virus suspension for one hour
4. Add 100µl from viral/ sample suspension .Place on a shaking table, 150rpm for 5 minutes.
5. Incubate (37C, 5% CO_2) for 1- days to allow the virus to take effect.
6. Make 2ml or more of MTT solution per 96 well plate at 5mg/ml in PBS.
7. Add 20ul MTT solution to each well. Place on a shaking table, 150rpm for 5 minutes, to thoroughly mix the MTT into the media.
8. Incubate (37C, 5% CO_2) for 1-5 hours to allow the MTT to be metabolized.
9. Dump off the media. (dry plate on paper towels to remove residue if necessary.
10. Resuspend Formosan (MTT metabolic product) in 200ul DMSO. Place on a shaking table, 150rpm for 5 minutes, to thoroughly mix the formazan into the solvent.
11. Read optical density at 560nm and subtract background at 620nm. Optical density should be directly correlated with cell quantity.

Antimicrobial activity
The standardized disc - agar diffusion method[19] 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 fumigatm:*

Screening for the antimicrobial potential:
Preparation of tested compounds
The tested compounds were dissolved in dimethyl formamide (DMF) solvent and prepared in two concentrations; 100 and 50 mg/ml and then 10 ui 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 vortexes then processed.

Testing for anti-bacterial 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 ul) from the specific concentration of dissolved compounds and carefully placed on surface of each inoculated plate. The plates were incubated in the upright position at 36°C for 24 hours. Three replicates were carried out for each extract against each of the test organism. Simultaneously, addition of the respective solvent instead of dissolved compound 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 $2.0xl0^5$ spore/ml, inoculum of 0.1 % suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes then the same procedure was followed as described above.

Standard references:
The antibiotic chloramphencol was used as standard reference in the case of Gram - negative bacteria. Cephalothin was used as standard reference in the case of Gram - positive bacteria and cyclohexlmide was used as standard reference in the case of yeasts and fungi.

REFERENCES

[1] Gravatt G. L, Baguley C, Wilson W. R. *J. Med. Chem*., **1994**, 37, 4338.
[2] Tempest P, Ma V, Thomas S, Hua Z, Kelly M.G, Hulme C., *Tetrahedron Lett*., **2001,** 42, 4959-4962.
[3] Trivedi R, De S. K, Gibbs R. A, *J. Mol. Catal. A. Chem*., **2005**, 245, 8-11.
[4] Divaeva L. N, Kuzmenko T. A, Morkovnik A. S, Komissarov V. N, *Chem. Hetero cyclic Compds*., **2006**, 42, 463-468.
[5] Davoodnia A, Roshani M, Saleh Nadim E, Bakavoil M, Tavakoli Hoseini N, *Chin. Chem. Lett.,* **2007**, 18, 1327-1330.
[6] VinodkumarVaidya R. S. D, Siva Kumar B.V, Bhise U.N, Bhirud S.B, Mashelkar U.C, *ARKIVOC. Xiv***, 2008,** 37-49.
[7] Sutha S, Shobana S, Dharmaraja J, Selvaraj S, *Spectro chim Acta A Mol Biomol. Spectrosc.,* **2013**,107C ,117-132.
[8] Reddy V. B, Singla R.K, Bhat V, Shenoy G, *Asian J. Resear Chem.*, **2009**, 2, 162-167.
[9] Micco I, Nencini A, Quinn J, Bothmann H, Ghiron C, Padova A. and Papini S, *Bioorg. Med. Chem.*, **2008**, 16, 2313-2328.
[10] Glal Sh. A, Abd El-All A. S, Abdallah M. M, El-Diwani H. I, *Bioorg. Med. Chem. Lett.*, **2009**, 19, 2420-2428.
[11] Glal Sh. A, Abd El-All A. S, Hegab K.H, Magd-El-Din A. A, Youssef N.S, El-Diwani H. I, *Eur. J. Med. Chem.*, **2010**, 45, 3035-3046.
[12] Saydin A, Beis K.D, Can D, *Pharmazie.*, **2003**, 58, 405-408.
[13] Rubbiani R, Kitanovic I, Hamed A, Can S, Kitanovic A, Onambele L, Stefanooulou M, Goldmacher Y, Sheldrick W, Wolber G, Pork A, Welof S, Ott I, *J. Med. Chem.*, **2010,** 53, 8608-8618.
[14] Kumar D, Jacob M.R, Reynolds M. B, Kerwin S.M, *Bioorg. Med.Chem*., **2002**, 10, 3997-4004.
[15] Suthakaran R, Nagarajan G, Balasubramaniam V, Suganthi K, Kannan M, Velrajan G, *Indian J. Heter. Chem.*, **2005**, 15, 173-176.
[16] Ishida T, Suzuki T, Hirashima S, MIzutani K, Adachi T, Hashimoto H, *Bioorg. Med. Chem. Lett.*, **2006**, 16, 1859-1863.
[17] Neef D. K, Lee-Dutra A, Blevitt J.M, Axe F.U, Hack M.D, Buma J.C, Rynberg R, Brunmark A, Karlsson L, Breitenbucher G, *Bioorg. Med. Chem. Lett.*, **2007**, 17, 6467-6471.
[18] Arienti K. L, Brunmark A, Axe F.U, Mcclure K, Lee A, Blevitt J, Neef D. K, Hung L, Crawford S, Pandit C.R, Karlsson L, Breitenbucher J.G, *J. Med. Chem.*,**2005**, 48,1873-1885.
[19] Joseph E. P, Bonnefous C, Symons K.T, Nguyen P.M, Sablad M, Rozenkrants N, Zhang Y, Wang L, Yazdani N, Shiau A. K, Noble S. A, Rix P, Rao T.S, Hassig C.A, Smith N.D, *J. Med. Chem.*,**2010**, 53,7739-7755.
[20] Ingle R.G, Magar D.D, *Heterocyclic* ,**2011**, 22, IJDRT, 1, 26-32.
[21] Scates A.C, Doraiswamy P.M, *Ann 23. Pharmacother*, **2000**, 34, 1302-1312.

[22] David B, Buis C, Mayers A, *Expert Rev. Neurother*, **2002**, 2, 717-724.
[23] Sharma M.C, Kohli D.V, Sharma S, Sharma A.D, *Der Pharmacia Sinica* , **2010**, 1, 104-115.
[24] Bharat S, Bhatta D, Panda P.K, Mishra P, Pradhan D, *Indian J. Pharm.* **2008**, 28.
[25] Rajasekaran S, Gopalkrishna R, Chatterjee A, *IJDDR* ,**2012**, 4,303-309.
[26] Khattab M, Galal Sh. A, Ragab F.A. F, El-Diwani H.I, *Res. Chem. Intermed.* **2013**, 39,2917.
[27] Gullapelli K, Bhaskar P, Muralikrishna T, Brahmeshwari G, *Int. J. Pharm. and biological Sci*. **2014**, 4(3), 59.
[28] Darwish E.S, *Sci.World. J* , **2014**, 1 .
[29] Sakurai A, Midorikawa H, Hashimoto Y, *Bull. Chem. Soc. Japan,* **1970**, 43, 2925-2933.
[30] Bauer A.W, Kirby W.M, Sherris J.C, Turk M, *Amer. J. Clinical. Pathology* , **1966**, 45(4), 493-496.