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Synthesis of novel cyanoacetamides derivatives and their urease inhibition studies

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ABSTRACT

The present study reports a convenient approach for the synthesis of cyanoacetamide based derivatives (7-27) *via* two-step process involving Knoevenagel reaction, followed by three component reaction to avail desired compounds. All the synthesized compounds were obtained in good to excellent yield and extensively characterized employing ^{1}H NMR, ^{13}C NMR, mass spectrometry and physical parameters. Further, these compounds were screened for urease inhibition. All of the synthesized compounds exhibited good to excellent urease activity notably compound 15 and 19 showed excellent urease inhibition activity with IC_{50} value ~17.34 µg/mL and 36.75 µg/mL in comparison to thiourea (used as standard) having IC_{50} value ~27.5 µg/mL.

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1. Introduction

Cyanoacetamides are highly reactive compounds and are utilized mainly as reactants, reaction intermediates or synthons to attain variety of organic compounds. Cyano acetamide based moieties such as phenyl-4,6-dimethyl-3-cyano-2-pyridones (**1**) and phenyl-4,5,6-trimethyl-3-cyano-2-pyridones (**2**) (Figure 1) and many others has interesting biological [1] and pharmaceutical applications such as potent anti-microbial [2,3] and were found useful as herbicidal, plant growth regulators and also active against neoplastic disorder and used as kynurenine-3-hydroxykase inhibitors [4,5], anti-inflammatory, antiviral, antibacterial [6], antitumor, neoplasm inhibitory [7], tyrosine kinase inhibitory, and analgesic properties, and for the treatment of disorders related to vasculogenesis [1,2]. The synthetic utility of *N*-aryl and/or heteryl cyanoacetamides in various organic heterocycles reveals their potential in evolving better chemotherapeutic agents [8,9].

Previously, we have reported various analogues based upon barbituric acid/thiobarbituric acid and proposed pharamacophores for biological activities such as urease inhibition studies [10,11], which provoked further to look for other valuable functionalities which could be incorporated on to potent nucleus. Therefore, 2-cyano-3-phenylprop-2-enamide derivatives prepared by Knoevenagel condensation of various aromatic and heterocyclic aldehydes with cyanoacetamide are further condensed with barbituric acid, *N,N*-dimethyl barbituric acid, thiobarbituric acid, malononitrile and 4-hydroxy coumarin moieties.

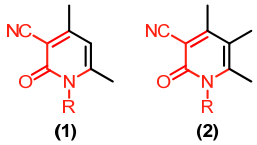

Figure 1. Cyanoacetoamide based pyridones.

2. Experimental

2.1. Instrumentation

All the synthesized compounds were purified and their physical parameters were recorded.

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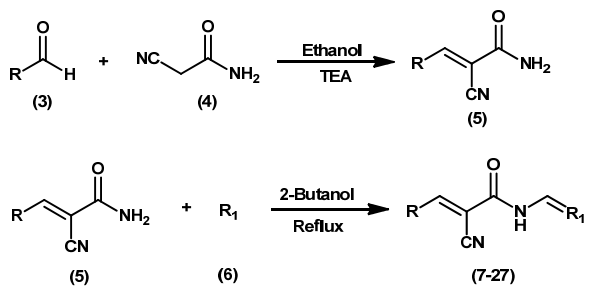

R= 3,4-dimethoxybenzaldehyde, 3-methoxy-4-hydroxybenzaldehyde, Furan-2-carbaldehyde, 2-Thiophenecaraldehyde, 3,4,5-trimethoxybezaldehyde

R_1= Barbituric acid, thiobarbituric acid, 1,3-dimethyl barbituric acid, 4-hydroxycoumrin, Malononitrile

Scheme 1

Melting points were determined with an Electrothermal 9100 apparatus and were uncorrected. Elemental analysis was performed using a Costech ECS 4010 CHNS-O analyzer at analytical laboratory of HEJ research institute university of Karachi. Mass spectra were recorded on a FINNIGAN-MAT 8430 mass spectrometer operating at an ionization potential of 70 eV. IR spectra were recorded on a Shimadzu IR-470 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on BRUKER DRX-500 AVANCE spectro-meter at 500.1 and 125.8 MHz, respectively. ^{1}H and ^{13}CNMR spectra were obtained on solution in DMSO-d_6 using TMS as internal standard. Thin layer chromatography was performed with Merck silica gel 60, 230-400 mesh.

2.2. Synthesis of novel derivatives of cyanoacetamides (7-27)

General procedure: Freshly prepared 2-cyano-3-phenylprop-2-enamide (**5**) derivatives (0.3 moles) were added to a stirring mixture of triethyl orthoformate (TEF) (0.3 moles), active methylene component (0.3 moles) in 2-butanol and the reaction mixture was refluxed till the completion of the reaction monitored by TLC (Scheme 1, Figure 2). The reaction was stopped and the hot precipitate were filtered off and further purified by extensive washing with boiling ethanol. The desired compounds were carefully dried and weighed.

2-Cyano-3-(3, 4-dimethoxyphenyl)-N-[(2, 4, 6-trioxotetra hydropyrimidin-5(2H)-ylidene)methyl]prop-2-enamide (**7**): Yield: 89%. Color: Light yellow. M.p.: 307-310 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.44 (d, 1H, *J* = 11.7 Hz, =CNH-), 11.47 (s, 1H, NH, Barbituric acid), 11.30 (s, 1H, NH Barbituric acid), 8.48 (d, 1H, *J* = 11.7 Hz, =CHNH-), 8.46 (s, 1H, Ar-CH=C(CN)CO-), 8.27 (s, 1H, Ar-H), 7.82 (d, 1H, *J* = 7.5 Hz, Ar-H), 7.22 (d, 1H, *J* = 8.4 Hz, Ar-H), 3.89 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 165.9 (CO), 163.5 (CO), 162.6 (CO), 160.2 (CO), 155.8 (CH), 154.3 (C), 150.5 (C), 148.8 (C), 146.2 (CH), 127.6 (CH), 116.4 (C); 113.2 (CH), 112.1 (CH), 100.3 (C), 98.7 (C), 56.0 (C), 55.4 (C). MS (EI, *m/z* (%): 370 (M^+, 89), 232 (17), 216 (100), 188 (31), 173 (13), 158 (11). Anal. calcd. for $C_{17}H_{14}N_4O_6$: C, 55.14; H, 3.81; N, 15.13. Found: C, 54.77; H, 3.48; N, 16.17%.

2-Cyano-3-(3, 4-dimethoxyphenyl)-N-[(4, 6-dioxo-2-thioxo tetrahydropyrimidin-5(2H)-ylidene)methyl]prop-2-enamide (**8**): Yield: 90%. Color: Light yellow. M.p.: 236-238 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.56 (s, 1H, NH Barbituric acid), 12.44 (d, 1H, *J* = 9.9 Hz, =CHNH-), 11.39 (s, 1H, NH Barbituric acid), 8.50 (d, 1H, *J* = 9.9 Hz, =CHNH-), 8.18 (s, 1H, Ar-CH=C(CN)CO-), 8.09 (s, 1H, Ar-H), 7.83 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.23 (d, 1H, *J* = 8.4 Hz, Ar-H), 3.89 (s, 3H, -OCH_3), 3.82 (s, 3H, -OCH_3). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 178.6 (CS), 176.4 (CO), 163.7 (CO), 160.8 (CO), 156.0 (CH), 154.4 (C), 150.5 (CH), 148.8 (C), 127.7 (CH), 125.4 (C), 116.4 (CH); 113.4 (C), 112.2 (CH), 102.9 (C), 98.6 (C), 56.0 (C), 55.5 (C). MS (EI, *m/z* (%)): 386 (M^+,90), 216 (100), 188 (19), 173(8.3), 156 (7), 130(5), 116.0 (4.1), 102.0 (3.0). Anal. calcd. for $C_{17}H_{14}N_4O_5S$: C, 52.84; H, 3.65; N, 14.50; S, 8.30. Found: C, 52.3; H, 3.48; N, 13.97; S, 8.19%.

2-Cyano-3-(3, 4-dimethoxyphenyl)-N-[(2, 4-dioxo-2H-chro men-3(4H)-ylidene)methyl] prop-2-enamide (**9**): Yield: 85%. Color: Light green. M.p.: 266-268 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 13.37 (d, 1H, *J* = 11.4 Hz, =CHNH-), 8.79 (d, 1H, *J* = 10.8 Hz, =CHNH-), 8.42 (s, 1H, Ar-CH=C(CN)CO-), 8.02 (d, 1H, *J* = 7.2 Hz, ArH), 7.83 (s, 1H, ArH), 7.77 (d, 2H, *J* = 7.2 Hz, ArH), 7.40 (d, 2H, *J* = 7.5 Hz, ArH), 7.02 (d, 1H, *J* = 8.4 Hz, ArH), 2.48 (s, 6H, OCH_3). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 161.6 (CO), 160.9 (CO), 156.6 (CH), 154.8 (CO), 154.1 (2C), 151.6 (CH), 149.8 (2C), 148.0 (CH), 136.7 (CH), 128.2 (CH), 126.3 (CH), 124.9 (CH), 123.1 (C), 117.5 (CH), 116.7 (CH), 114.9 (CH), 104.0 (C), 96.9 (C), 55.7 (C), 54.6 (C). MS (EI, *m/z* (%)): 404 (M^+, 52), 216 (100), 202 (31.0) 188(16), 169.9 (17). Anal. calcd. for $C_{22}H_{16}N_2O_6$: C, 65.34; H, 3.99; N, 6.93. Found: C, 65.89; H, 3.54; N, 6.37%.

2-cyano-3-(3, 4-dimethoxyphenyl)-N-((1,3-dimethyl-2, 4, 6-trioxotetrahydropyrimidin-5(2H)-ylidene)methyl) acrylamide (**10**): Yield: 96%. Color: Pink. M.p.: 294-296 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.44 (d, 1H, *J* = 9.9 Hz, =CHNH-), 8.50 (d, 1H, *J* = 9.9 Hz, =CHNH-), 8.41 (s, 1H, Ar-CH=C(CN)CO-), 8.29 (s, 1H, ArH), 7.57 (d, 1H, *J* = 8.4 Hz, ArH), 7.09 (d, 1H, *J* = 8.4 Hz, ArH), 3.89 (s, 6H, OCH_3), 3.82 (s, 6H, NCH_3). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 173.7 (CO), 171.9 (CO), 165.6 (CO), 162.3 (CO), 159.5 (CH), 156.4 (C), 153.6 (CH),149.1 (C), 129.1 (CH), 127.4 (C), 119.0 (CH), 116.4 (C), 112.1 (CH), 107.9 (C), 99.9 (C), 56.0 (C), 55.5 (C), 46.0 (C), 41.5 (C). MS (EI, *m/z* (%)): 398 (M^+, 52), 230 (9.1), 216 (100), 202 (31.0), 188(16), 173 (13), 169.9 (17), 158 (11).

2-Cyano-3-(4-hydroxy-3-methoxyphenyl)-N-[(2, 4, 6-trioxo tetrahydropyrimidin-5(2H)-ylidene) methyl] prop-2-enamide (**11**): Yield: 73%. Color: Yellow. M.p.: 259-260 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.43 (d, 1H, *J* = 11.7 Hz, =CHNH-), 11.46 (s, 1H, NH Barbituric acid), 11.29 (s, 1H, NH Barbituric acid), 10.47 (s, 1H, Phenolic),8.48 (d, 1H, *J* = 11.7 Hz, =CHNH-), 8.41 (s, 1H, Ar-CH=C(CN)CO-), 8.33 (s, 1H, ArH), 7.72 (d, 1H, *J* = 7.8 Hz, ArH), 7.00 (s, 1H, ArH), 3.83 (s, 3H, OCH_3). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 166.0 (CO), 163.8 (CO), 162.7 (CO), 160.4 (CO), 156.0 (CH), 153.8 (C), 150.5 (C), 148.6 (CH), 146.3 (C), 128.0 (CH), 123.0 (C), 116.3 (CH), 114.5 (CH), 100.0 (C), 97.6(C), 55.6 (C). MS (EI, *m/z* (%)): 356.0 (M^+, 42), 216 (17), 202 (78), 170 (100), 140.0 (42), 114 (42), 103 (19), 76 (24).

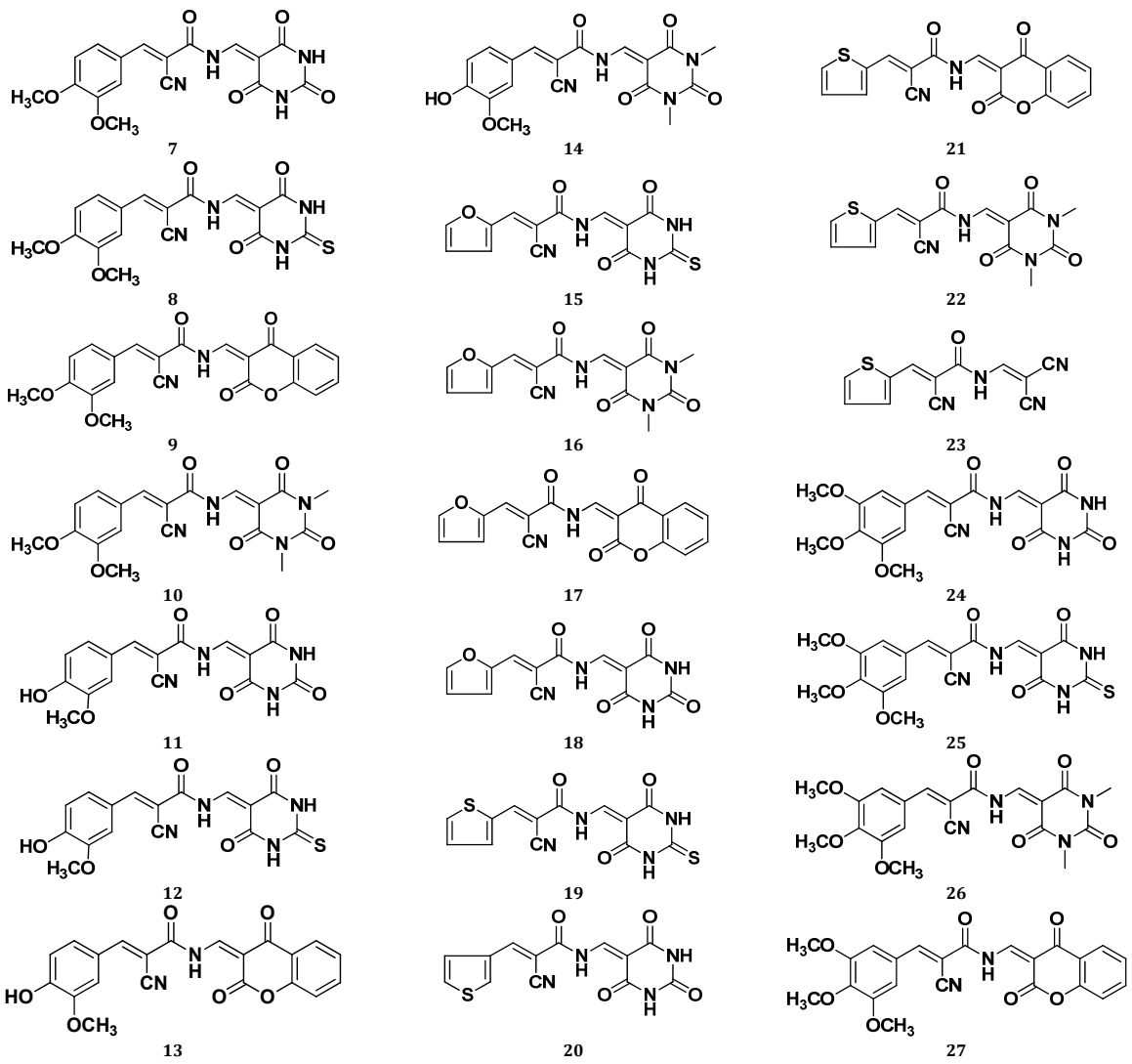

Figure 2. Compounds **7**-**27** synthesised via one pot multicomponent reaction.

2-Cyano-N-[(4,6-dioxo-2-thioxotetrahydropyrimidin-5(2H)-ylidene)methyl]-3-(4-hydroxy-3-methoxyphenyl)prop-2-enamide (**12**): Yield: 91%. Color: Yellow. M.p.: 264-265 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.43 (d, 1H, *J* = 11.7 Hz, =CHNH-), 11.80 (s, 1H, NH Barbituric acid), 11.74 (s, 1H, NH Barbituric acid), 10.80 (s, 1H, Phenolic), 9.44 (s, 1H, ArH), 8.50 (d, 1H, *J* = 12.0 Hz, =CHNH-), 8.41 (s, 1H, Ar-CH=C(CN)CO-), 7.73 (dd, 1H, *J* = 8.4 Hz, ArH), 6.99 (d, 1H, *J* = 9.1 Hz, ArH), 3.83 (s, 3H, OCH_3). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 178.6 (CO), 177.8 (CO), 163.8 (CO), 162.2 (C), 160.8 (C), 158.8 (CH), 156.2 (CH), 153.9 (C), 147.3 (CH), 128.1 (CH), 123.0 (C), 116.3 (CH), 114.6 (C), 100.8 (C), 96.9 (C), 55.6 (CH). MS (EI, *m/z* (%)): 372 (M^+, 12), 202 (23), 171 (100), 142 (12), 113 (15). Anal. calcd. for $C_{16}H_{12}N_4O_5S$: C, 51.61; H, 3.25; N, 15.05. Found: C, 50.97; H, 3.21; N, 15.35%.

2-Cyano-N-[(2,4-dioxo-2H-chromen-3(4H)-ylidene)methyl]-3-(4-hydroxy-3-methoxyphenyl)prop-2-enamide (**13**): Yield: 92%. Color: Light orange. M.p.: 260-262 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 13.37 (d, 1H, *J* = 11.4 Hz, =CHNH-), 10.86 (s, 1H, Phenolic), 8.78 (d, 1H, *J* = 10.8 Hz, =CHNH-), 8.47 (s, 1H, Ar-CH=C(CN)CO-), 8.02 (d, 1H, *J* = 7.2 Hz, ArH), 7.83 (s, 1H, ArH), 7.77 (d, 2H, *J* = 7.2 Hz, ArH), 7.40 (d, 2H, *J* = 7.5 Hz, ArH), 7.02 (d, 1H, *J* = 8.4 Hz, 1H, ArH), 3.84 (s, 3H, OCH_3). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 161.6 (CO), 160.9 (CO), 156.6 (CH), 154.8 (CO), 154.1 (2C), 151.6 (CH), 149.8 (2C), 148.0 (CH), 136.7 (CH), 128.2 (CH), 126.3 (CH), 124.9 (CH), 123.1 (C), 117.5 (CH), 116.7 (CH), 114.9 (CH), 104.0 (C), 96.9 (C), 55.7 (C). MS (EI, *m/z* (%)): 390 (M^+, 26), 216 (27), 202 (65), 170.0 (100), 142 (34), 114 (40), 92 (30), 76 (22). Anal. calcd. for $C_{21}H_{14}N_2O_6$: C, 64.62; H, 3.62; N, 7.18. Found: C, 64.24; H, 3.79; N, 7.23%.

2-Cyano-N-[(1,3-dimethyl-2,4,6-trioxotetrahydropyrimidin-5(2H)-ylidene) methyl]-3-(4-hydroxy-3-methoxyphenyl)prop-2-enamide (**14**): Yield: 75%. Color: Yellow. M.p.: 235-237 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.42 (d, 1H, *J* = 11.4 Hz, =CHNH-), 10.81 (s, 1H, Phenolic), 8.59 (d, 1H, *J* = 12.0 Hz, =CHNH-), 8.44 (s,1H, Ar-CH=C(CN)CO-), 7.80 (s, 1H, ArH), 7.75 (d, 1H, *J* = 8.4 Hz, ArH), 7.50 (d, 1H, *J* = 8.4 Hz, ArH), 3.83 (s, 3H, OCH_3), 3.32 (s, 6H, NCH_3). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 178.3 (CO), 176.1 (CO), 163.5 (CO), 161.9 (CO), 159.5 (CH), 157.6 (C), 156.1 (CH),152.3 (C), 131.2 (CH), 127.4 (C), 122.0 (CH), 119.2 (C), 117.3 (CH), 110.7 (C), 99.9 (C), 53.9 (C), 51.5 (C), 42.9 (C). MS (EI, *m/z* (%)): 384.0 (M^+, 56), 216 (36), 202 (89.9), 170 (100), 142 (44), 129 (15), 114 (56), 103 (25), 77 (14). Anal. calcd. for $C_{18}H_{16}N_4O_6$: C, 56.25; H, 4.20; N, 14.58. Found: C, 55.87; H, 4.08; N, 14.17%.

*2-Cyano-N-[(4,6-dioxo-2-thioxotetrahydropyrimidin-5(2H)-ylidene) methyl]-3-(furan-2-yl)prop-2-*enamide (**15**): Yield: 83%. Color: Yellow. M.p.: 270-271 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.57 (s, 1H, NH Barbituric acid), 12.45 (d, 1H, *J* = 11.4 Hz, =CHNH-), 11.41 (s, 1H, NHBarbituric acid), 8.47 (d, 1H, *J* = 11.7 Hz, =CHNH-), 8.32 (d, 2H, *J* = 4.8 Hz, ArH), 8.18 (s, 1H, Ar-CH=C(CN)CO), 7.54 (d, 1H, *J* = 3.3 Hz, ArH). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 178.6 (CS), 176.4 (CO), 163.7 (CO), 160.9 (CO), 152.3 (CH), 148.6 (C), 147.1 (CH), 139.6 (CH), 137.4 (C), 127.6 (CH), 114.9 (CH), 102.9 (C), 97.0 (C). MS (EI, *m/z* (%)): 316.0 (M^+, 8), 146 (100), 118 (18), 90 (60), 63.0 (12). Anal. calcd. for $C_{13}H_8N_4O_4S$: C, 49.37; H, 2.55; N, 17.71. Found: C, 50.04; H, 2.44; N, 17.29%.

2-Cyano-N-[(1,3-dimethyl-2,4,6-trioxotetrahydropyrimidin-5(2H)-ylidene) methyl]-3-(furan-2-yl)prop-2-enamide (**16**): Yield: 83%. Color: Brown. M.p.: 263-265 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.42 (d, 1H, *J* = 12.0 Hz, =CHNH), 8.56 (d, 1H, *J* = 12.0 Hz, =CHNH-), 8.42 (s, 1H, Ar-CH=C(CN)CO), 8.33 (d, 2H, *J* = 3.6 Hz, ArH), 7.65 (d, 1H, *J* = 3.6 Hz, ArH), 3.32 (s, 6H, NCH_3). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 172.9 (CO), 173.7 (CO), 166.17 (CO), 162.9 (CO), 157.0 (CH), 152.7 (C), 149.3 (CH), 143.6 (CH), 139.4 (C), 137.1 (CH), 124.4 (CH), 112.7 (C), 100.7 (C), 42.7 (C), 40.0 (C). MS (EI, *m/z* (%)): 328.0 (M^+, 15), 146 (100), 118 (15), 90 (25). Anal. calcd. for $C_{15}H_{12}N_4O_5$: C, 54.88; H, 3.68; N, 17.07. Found: C, 54.77; H, 3.48; N, 17.17%.

2-Cyano-N-[(2,4-dioxo-2H-chromen-3(4H)-ylidene)methyl]-3-(furan-2-yl) prop-2-enamide (**17**): Yield: 78%. Color: Green yellow. M.p.: 240-243 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 13.36 (d, 1H, *J* = 11.7 Hz, =CHNH-), 8.76 (d, 1H, *J* = 12.0 Hz, =CHNH-), 8.39 (s, 1H, Ar-CH=C(CN)CO), 8.35 (d, 1H, *J* = 7.2 Hz, ArH), 8.03 (d, 1H, *J* = 7.8 Hz, ArH), 7.79 (d, 1H, *J* = 7.2 Hz, ArH), 7.69 (d, 1H, *J* = 3.3 Hz, ArH), 7.39 (t, 2H, *J* = 7.9 Hz, ArH), 6.95 (d, 1H, *J* = 1.8 Hz, ArH). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 151.6 (2CO), 151.4 (CO), 149.6 (C), 148.7 (C), 140.0 (2CH), 136.7 (CH), 136.4 (CH), 127.5 (CH), 126.3 (2C), 124.9 (CH), 119.6 (CH), 117.6 (C), 117.5 (C), 115.4 (C), 115.0 (C). MS (EI, *m/z* (%)): 334.0 (M^+, 26), 216 (3), 188.1 (14), 146.0 (100), 121.0 (24), 90 (28), 63.0 (34). Anal. calcd. for $C_{18}H_{10}N_2O_5$: C, 64.67; H, 3.02; N, 8.38. Found: C, 64.54; H, 3.09; N, 8.23%.

2-Cyano-3-(furan-2-yl)-N-[(2, 4 ,6-trioxotetrahydropyrimidin-5(2H)-ylidene)methyl] prop-2-enamide (**18**): Yield: 91%. Color: Gray. M.p.: 251-253 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.43 (d, 1H, *J* = 11.4 Hz, =CHNH), 11.48 (s, 1H, NH barbituric acid), 11.31 (s, 1H, NH barbituric acid), 8.45 (d, 1H, *J* = 11.7 Hz, =CHNH-), 8.38 (s, 1H, Ar-CH=C(CN)CO), 8.30 (d, 1H, *J* = 3.9 Hz, ArH), 7.62 (d, 1H, *J* = 3.6 Hz, ArH), 6.92 (m, 1H, ArH). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 166.0 (CO), 164.0 (CO), 162.6 (CO), 160.1 (CO), 151.2 (CH), 148.6 (CH), 146.1 (C), 139.6 (CH), 135.9 (CH), 126.9 (CH), 121.4 (C), 115.0 (C), 100.4 (C). MS (EI, *m/z* (%)): 300.0(M^+ 6), 146 (87), 90 (71), 63 (100). Anal. calcd. for $C_{13}H_8N_4O_5$: C, 52.01; H, 2.69; N, 18.66; O, 26.65; Found: C, 51.91; H, 2.79; N, 18.76.

2-Cyano-N-[(4,6-dioxo-2-thioxotetrahydropyrimidin-5(2H)-ylidene)methyl]-3-(thiophen-3-yl)prop-2-enamide (**19**): Yield: 82%. Color: Gray. M.p.: 246-250 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.57 (s, 1H, NH Barbituric acid), 12.43 (d, 1H, *J* = 11.8 Hz, =CHNH-), 11.42 (s, 1H, NH Barbituric acid), 8.47 (d, 1H, *J* = 11.7 Hz, =CHNH-), 8.32 (s, 1H, Ar-CH=C(CN)CO), 8.16 (d, 2H, *J* = 4.8 Hz, ArH), 7.48 (s, 1H, ArH). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 178.6 (CS), 176.4 (CO), 163.7 (CO), 160.8 (CO), 148.6 (CH), 147.2(CH), 143.5(C), 141.8 (CH), 138.8 (CH), 135.9 (C), 129.3 (CH), 115.8 (C), 100.9 (C). MS (EI, *m/z* (%)): 332.0 (M^+, 15), 318.0 (64), 306.0 (26), 284.0 (13), 265.0 (41), 162.0 (100), 134.0 (50). Anal. calcd. for $C_{13}H_8N_4O_3S_2$: C, 46.98; H, 2.43; N, 16.86. Found: C, 46.63; H, 2.31; N, 16.53%.

2-Cyano-3-(thiophen-3-yl)-N-[(2,4,6-trioxotetrahydropyrimidin-5(2H)-ylidene) methyl] prop-2-enamide (**20**): Yield: 84%. Color: Yellowish. M.p.: 261-263 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.44 (d, 1H, *J* = 11.7 Hz, =CHNH-), 11.48 (s, 1H, NH Barbituric acid), 11.44 (s, 1H, NHB arbituric acid), 8.46 (s, 1H, Ar-CH=C(CN)CO), 8.33 (s, 1H, =CHNH-), 8.16(d, 2H, *J* = 4.8 Hz, ArH), 7.43 (d, 1H, *J* = 3.3 Hz, ArH). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 166.0 (CO), 164.6 (CO), 162.6 (CO), 160.0 (CO), 148.8(CH), 143.5(CH), 138.86 (CH), 134.9 (CH), 128.6 (C), 116.7 (C), 102.0(C), 100.3 (C), 98.25 (C). MS (EI, *m/z* (%)): 316.1 (M^+, 10), 176.0 (15), 162.0 (100), 134.0 (51), 90 (38). Anal. calcd. for $C_{13}H_8N_4O_4S$: C, 49.37; H, 2.55; N, 17.71. Found: C, 49.23; H, 2.11; N, 17.83%.

2-Cyano-N-[(2,4-dioxo-2H-chromen-3(4H)-ylidene)methyl]-3-(thiophen-3-yl)prop-2-enamide (**21**): Yield: 75%. Color: Light yellow. M.p.: 261-264 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 13.35 (d, 1H, *J* = 11.7 Hz, =CHNH-), 8.87 (s, 1H, Ar-CH=C(CN) CO), 8.39 (d, 1H, *J* = 7.8 Hz, =CHNH-), 8.20 (s, 1H, ArH), 8.02 (d, 1H, *J* = 7.2 Hz, ArH), 7.77 (m, 1H, ArH), 7.60 (d, 1H, *J* = 7.5 Hz, ArH), 7.50 (m, 2H, ArH), 7.36 (m, 1H, ArH). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 159.7 (CO), 154.7 (CO), 152.3 (CO), 151.4 (C), 149.0 (CH), 142.0 (C), 139.1 (CH), 136.7 (CH), 133.0 (CH), 132.3 (CH), 129.4 (C), 126.3 (C), 124.9 (CH), 124.0 (CH), 123.11 (CH), 116.6 (CH), 113.0 (C), 102.4 (C). MS (EI, *m/z* (%)): 350.0 (M^+, 5), 176 (24), 162.0 (100), 134.0 (60), 121 (24), 90 (28), 63 (34). Anal. calcd. for $C_{18}H_{10}N_2O_4S$: C, 61.71; H, 2.88; N, 8.00. Found: C, 61.19; H, 2.35; N, 7.69%.

2-Cyano-N-[(1,3-dimethyl-2,4,6-trioxotetrahydropyrimidin-5(2H)-ylidene)methyl]-3-(thiophen-3-yl)prop-2-enamide (**22**): Yield: 89%. Color: Yellow. M.p.: 290-292 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.39 (d, 1H, *J* = 11.7 Hz, =CHNH-), 8.81 (s, 1H, Ar-CH=C(CN)CO), 8.57 (d, 1H, *J* = 11.7 Hz, =CHNH), 8.30 (d, 1H, *J* = 4.8 Hz, ArH), 8.18 (s, 1H, ArH), 7.41 (d, 1H, *J* = 4.5 Hz, ArH), 3.21 (s, 6H, NCH_3). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 166.0 (CO), 164.6 (CO), 162.6 (CO), 160.0 (CO), 150.0(C), 148.5(CH), 143.5(CH), 141.6 (CH), 138.9 (CH), 135.9 (CH), 134.9 (C), 129.3 (C), 116.7 (C), 100.1 (C), 97.3 (C). MS (EI, *m/z* (%)): 344.0 (M^+, 6), 210.1 (9), 176.0 (24), 162.0 (100), 134 (65), 90.0 (31), 58 (35). Anal. calcd. for $C_{15}H_{12}N_4O_4S$: C, 52.32; H, 3.51; N, 16.27. Found: C, 51.92; H, 3.31; N, 16.07%.

2-Cyano-N-(2, 2-dicyanoethenyl)-3-(thiophen-3-yl)prop-2-enamide (**23**): Yield: 93%. Color: Yellow. M.p.: 269-271 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.39 (d, 1H, *J* = 11.7 Hz, =CHNH-), 8.71 (s, 1H, Ar-CH=C(CN)CO), 8.28 (d, 1H, *J* = 11.7 Hz, =CHNH-), 7.63 (s, 1H, ArH), 7.37 (m, 1H, ArH), 7.29 (m, 1H, ArH). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 164.0 (CO), 162.5 (C), 153.4 (CH), 143.5 (CH), 140.4 (CH), 138.6 (CH), 137.6 (C), 135.3 (C), 134.8 (C), 129.2 (CH), 116.6 (C), 114.3 (C). MS (EI, *m/z* (%)): 254.0 (M^+, 1), 178.0 (27), 162.0 (15), 133.0 (66), 108.9 (79), 90 (17), 58.0 (100). Anal. calcd. for $C_{12}H_6N_4OS$: C, 56.68; H, 2.38; N, 22.03. Found: C, 55.98; H, 2.39; N, 22.73%.

2-Cyano-3-(3, 4, 5-trimethoxyphenyl)-N-[(2, 4, 6-trioxotetrahydropyrimidin-5(2H)-ylidene) methyl] prop-2-enamide (**24**): Yield: 80%. Color: Light pink. M.p.: 300-302 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.44 (d, 1H, *J* = 11.5 Hz, =CHNH-), 11.49(s, 1H, NH Barbituric acid), 11.31 (s, 1H, NH Barbituric acid), 8.47 (s, 1H, Ar-CH=C(CN)CO-), 8.45 (d, 1H, *J* = 11.5 Hz, =CH-NH), 7.54 (s, 2H, ArH), 3.83 (s, 6H, OCH_3), 3.79 (s, 3H, OCH_3). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 165.9 (CO), 164.4 (CO), 162.5 (CO), 159.9 (CO), 155.8 (CH), 152.9 (C), 150.2 (C), 148.8 (CH), 146.0 (CH), 126.4 (C), 116.0 (C), 109.3 (CH), 107.9 (C), 101.0 (C), 100.5 (C), 99.9 (C), 67.0 (C), 60.40 (C), 56.0 (C). MS (EI, *m/z* (%)): 400.1(M^+, 4), 262 (15), 215 (11), 181 (35), 155 (35), 144 (13), 128 (13), 117(16), 105 (15), 90(24), 85 (63). Anal. calcd. for $C_{18}H_{16}N_4O_7$: C, 54.00; H, 4.03; N, 13.99. Found: C, 53.34; H, 3.89; N, 13.78%.

2-Cyano-N-[(4,6-dioxo-2-thioxotetrahydropyrimidin-5(2H)-ylidene)methyl]-3-(3, 4, 5-trimethoxyphenyl)prop-2-enamide (**25**): Yield: 95%. Color: Light orange. M.p.: 228-232 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.57 (s, 1H, NH Barbituric acid), 12.44 (d, 1H, *J* = 10.2 Hz, =CHNH-), 11.41 (s, 1H, NH Barbituric acid), 8.49 (s, 1H, Ar-CH=C(CN)CO), 8.45 (d, 1H, *J* = 6.6 Hz, =CH-NH), 7.54 (s, 2H, ArH), 3.80 (s, 9H, OCH_3). ^{13}C NMR (300MHz, DMSO-d_6, δ, ppm): 178.6 (CS), 176.4 (CO), 163.7 (CO), 160.7 (CO), 159.8 (C), 156 (CH), 152.8 (C), 148.5 (CH), 147.0 (CH), 142.9 (C), 126.4 (C), 116.0 (C), 109.3 (H), 101.1

Table 1. *In-vitro* urease inhibition of compound **7-27** *.

Compound	Inhibition (%) at0.5 mM ± S.E.M.	IC_{50}, µM	Compound	Inhibition (%) at0.5 mM ± S.E.M.	IC_{50}, µM
7	31.38±0.24	-	18	27.91±0.72	-
8	41.16±0.32	-	19	81.71±0.61	36.75±0.05
9	18.15±0.81	-	20	83.14±0.53	195.14±0.11
10	8.12±0.41		21	34.44±0.21	-
11	23.10±0.89	-	22	1.65±0.24	-
12	-	-	23	53.11±0.35	-
13	24.88±0.61	-	24	25.12±0.87	-
14	5.74±0.78	-	25	37.63±0.13	-
15	88.61±0.24	179.81±0.08	26	13.11±0.75	
16	91.11±0.59	17.34±0.01	27	7.51±0.87	-
17	34.57±0.63	-			

* Thiourea used as standard (IC_{50} value of 27.5 µg/mL).

(C), 100.8 (C), 60.40 (C),60.43 (C), 56.0 (2C). MS (EI, *m/z* (%)): 416 (M^+ 4.17%), 161 (26.9), 196 (30), 224 (52), 371.2(100). Anal. calcd. for $C_{18}H_{16}N_4O_6S$: C, 51.92; H, 3.87; N, 13.45. Found: C, 51.04; H, 3.26; N, 13.88%.

2-Cyano-N-[(1,3-dimethyl-2,4,6-trioxotetrahydropyrimidin-5(2H)-ylidene) methyl]-3-(3, 4, 5-trimethoxyphenyl) prop-2-enamide (**26**): Yield: 83%. Color: Light orange. M.p.: 277-280 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.44 (d, 1H, *J* = 12.0 Hz, =CHNH-), 8.58 (d, 1H, *J* = 11.5 Hz, =CH-NH), 8.52 (s, 1H, Ar-CH=C(CN)CO-), 7.58 (s, 2H, ArH), 3.84 (s, 6H, OCH_3), 3.81 (s, 3H, OCH_3), 3.21 (s, 6H, CH_3). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 165.9 (CO), 164.4 (CO), 162.5 (CO), 159.9 (CO), 155.8 (CH), 152.9 (C), 150.2 (C), 148.8 (CH), 146.0 (CH), 126.4 (C), 116.0 (C), 109.3 (C), 107.9 (C), 101.0 (C), 100.5 (C), 99.9 (C) 60.40 (C), 56.0 (C). MS (EI, *m/z* (%)): 428 (M^+, 22), 246 (100), 215 (50), 188 (28), 161 (26). Anal. calcd. for $C_{20}H_{20}N_4O_7$: C, 56.07; H, 4.71; N, 13.08. Found: C, 55.37; H, 4.26; N, 13.09%.

2-Cyano-N-[(2,4-dioxo-2H-chromen-3(4H)-ylidene)methyl]-3-(3, 4, 5-trimethoxyphenyl)prop-2-enamide (**27**): Yield: 92%. Color: Yellow. M.p.: 260-263 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.57 (d, 1H, *J* = 10.2 Hz, =CHNH-), 8.49 (d, 1H, *J* = 11.7 Hz, =CH-NH), 8.41 (s, 1H, Ar-CH=C(CN)CO-), 7.98 (d, 1H, *J* = 7.2 Hz, ArH), 7.79 (s, 1H, ArH), 7.75 (d, 2H, *J* = 7.2 Hz, ArH), 7.46 (d, 1H, *J* = 7.5 Hz, ArH), 7.12 (d, 1H, *J* = 8.4 Hz, ArH), 3.84 (s, 6H, OCH_3), 3.83 (s, 3H, OCH_3). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 182.5 (CO), 164.1 (CO), 156.3 (CH), 154.7 (CO), 151.6 (CH), 136.6 (CH), 134.9 (CH), 133.0 (CH), 126.3 (CH), 125.0 (C), 124.8 (CH), 123.9 (C), 123.0 (CH), 122.7 (C), 117.5 (CH), 116.7 (CH),116.5 (C),116.1 (C),104.1 (C),96.9 (C), 55.3 (CH_3), 44.2 (CH_3), 43.1 (CH_3). Anal. calcd. for $C_{23}H_{18}N_2O_7$: C, 63.59; H, 4.18; N, 6.45. Found: C, 62.99; H, 4.36; N, 5.88%.

2.3. Urease inhibition assay

Urease inhibition of newly synthesized compounds was determined by the modified phenol-hypochlorite method [10-15] (Table 1). Urease inhibition curve of thiourea was used as reference at varying concentrations i.e., 100, 10, 1, 0.1 and 0.01 µM. The reaction mixtures comprising sample solution (2 µL), phosphate buffer (17 µL) and sodium nitroprusside (20 µL) were incubated at 37 °C for 2 h in well plates. Then Griess reagent (20 µL, 0.3% sulphanilic acid in glacial acetic acid and 0.1% Naphthyl ethylene diamine (NED): equal volumes of both solutions mixed just before use) was added to each well plate and the mixture kept at room temperature for 20 min to develop the color. The absorbance was measured at 528 nm by using an ELISA reader. The results were processed using Gen 5 software. All the reactions were performed in triplicate. Positive control (containing known antiurease) and control (containing all samples) were run in parallel. Negative control was used to calibrate the instrument. The % age antiurease activity was determined by using the Equation (1).

$$\% \text{ Antiurease activity} = \frac{(\text{Abs. of control} - \text{Abs. of sample})}{(\text{Absorbance of control})} \times 100 \quad (1)$$

3. Results and discussion

3.1. Synthesis

Synthesized cyanoacetamide based compounds **7**-**27** were obtained in good to excellent yield (59-87%) as colored precipitates from hot stirred reaction mixture via one pot multi-component reaction. All the compounds were recrystallized in ethanol and were extensively characterized by their physical, spectral (IR, ^{1}H NMR, ^{13}C NMR, Mass) and elemental analyses data to establish their structures. The biological activities such as antibacterial, antiurease and antioxidant were also carried out and structural activity relationship was explored.

A representative compound **7**'s structure elucidation is discussed. The ^{1}H NMR spectrum of compound **7** justified the proposed structure, which displayed the amide proton *(*=CHN*H*-) downfield as doublet (*J* = 11.7 Hz) at δ 12.44 ppm. The pyrimidine nonequivalent amide NH protons appeared as separate singlet at δ 11.30 ppm. The second NH appeared slightly downfield at δ 11.47 ppm due to the involvement of intermolecular hydrogen bonding between carbonyl oxygen of pyrimidine ring and methylidene amino function (N-H). The methylidene proton (=C*H*NH-) appeared as doublet at δ 8.48 ppm (*J* =11.7 Hz) and the olefinic proton (Ar-C*H*=C(CN)CO-) appeared as a singlet at δ 8.46 ppm. In the spectrum of compound ***7*** aromatic proton appeared downfield as a singlet at δ 8.27 ppm whereas other aromatic protons appeared as two separate doublets at δ 7.22 and 7.82 ppm. The methoxy protons appeared as two singlet at δ 3.89 and 3.82 ppm, respectively.

The ^{13}C NMR spectrum of compound **7** accounted 17 Cs, displayed two carbonyl carbons (=CCO) downfield at δ 165.9 and 163.5 ppm whereas (CNCCONH) and (NHCONH) at δ 162.6 and 160.18 ppm. The carbon (CONHCH=) display signals downfield at δ 155.8 ppm. The aromatic ring carbons (C-OCH_3) displayed signals at δ 154.3 and 150.6 ppm, respectively. The barbituric acid ring carbon (COCCO) exhibited signals at δ 148.8 ppm. The carbons Ar-CH=C(CN) CO) appeared at δ 146.2 ppm. The aromatic ring carbons were observed at δ 127.6, 113.2 and 112.2 ppm, respectively. The carbons Ar-CH=C(CN)CO and the ipso carbon next the group recorded signals at δ 116.4 and 100.3 and 98.7 ppm, whereas the methoxy carbons appeared at δ 56.0 and 55.4 ppm.

The mass spectrum of compounds **7** recorded molecular ion peak at 370 amu and rest of the fragmentation was in accordance with the proposed structure. Furthermore, elemental analysis was in agreement with the proposed structure with anal. calcd. for $C_{17}H_{14}N_4O_6$: C, 55.14; H, 3.81; N, 15.13. Found: C, 54.77; H, 3.48; N, 17.17%.

3.2. Anti-urease activity

The chemical nature of cyanoacetamide based compounds encouraged us to study the antiurease activities of our newly synthesized compounds. Antiurease activity was carried out

by using thiourea, with an IC_{50} value of 27.5 µg/mL, as the standard. The synthesized compounds **7-27** were examined for their urease inhibition activity at 0.5mM and IC_{50}µM concentration (Table 1, Figure 3). It was considered that all these compounds could have the ability to bind with the enzyme's active site and the synthesized compounds showed moderate to excellent urease inhibition. The compounds **16** and **19** proved to be the most potent urease inhibitor, with IC_{50} values of 17.34 µg/mL and 36.75 µg/mL, respectively. These values were comparable to the 27.5 µg/mL value of the thiourea (standard) whereas molecules **15** and **20** exhibited comparatively moderate antiurease activities.

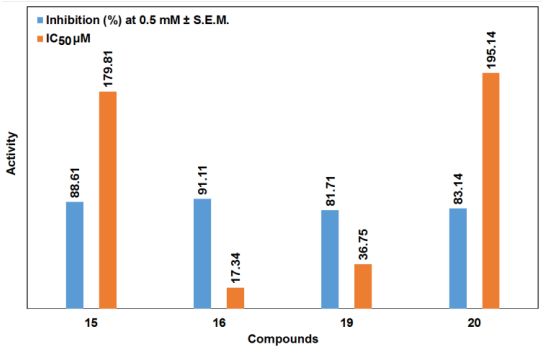

Figure 3. Most active urease inhibition shown by compound 15, 16, 19 and 20.

4. Conclusion

Various cyano acetamides based compounds were availed *via* three component-single pot reactions in good to excellent yield and extensively characterized. Among the 21 synthesized compounds, five of them exhibited moderate to excellent urease inhibition. All these active compounds compromises of barbituric acid, *N*,*N*-dimethyl barbituric acid and thiobarbituric acid moiety and these are already known for their potency for urease inhibition [12-15]. Compound **16** exhibited ~91% inhibition and IC_{50} value of 17 µg/mL which in comparison to standard thiourea is more potent, similarly, compound **19** exhibited 82% inhibition and IC_{50} value of 36µg/mL. In comparison to the rest of the synthesized compounds, compounds **16** and **19** have furan and thiophene ring, whereas in all other compounds substituted phenyl rings are present. These two five member rings containing O and S generating an extra pocket to interact with Ni which is so critical for the urease enzyme activity [17]. Therefore it is speculated that furan-CH=C(CN)CO- and thiophene-CH=C(CN) CO- are two new pharmacophore which can be employed to enhance urease inhibition activity.

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