A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index;
Bangladesh J Pharmacol 2016; 11: 894-902
ISSN: 1991-0088

New 1-octanoyl-3-aryl thiourea derivatives: Solvent-free synthesis, characterization and multi-target biological activities

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Article Info

Received: 2 August 2016
Accepted: 11 October 2016
Available Online: 14 November 2016

DOI: 10.3329/bjp.v11i4.29059

Cite this article:
Saeed A, Larik FA, Channar PA, Ismail H, Dilshad E, and Mirza B. New 1-octanoyl-3-aryl thiourea derivatives: solvent-free synthesis, characterization and multi-target biological activities. Bangladesh J Pharmacol. 2016; 11: 894-902.

Abstract

An efficient solvent-free synthesis of a 10-member library of octanoyl linked substituted aryl thioureas was accomplished successfully. The octanoyl isothiocyanate was freshly prepared in excellent yield and purity by the reaction of potassium thiocyanate with octanoyl chloride followed by removal of potassium chloride by filtration. The reaction of the latter with a series of ten different substituted anilines by stirring at 60-65°C lead to the formation of the title compounds. The *in vitro* antifungal activity of newly synthesized compounds was evaluated against *Aspergillus niger, A. flavus* and *Fusarium solani* strains of pathogenic fungi. Antibacterial assay was carried out against Gram positive (*Staphylococcus aureus, Micrococcus luteus*) and Gram negative bacterial strains *(Escherichia coli, Enterobacter aerogens)*. Furthermore, anti-oxidant potential and enzyme inhibition studies against α-amylase and butyryl cholinestrase were performed. The results obtained indicated moderate to excellent activities of most of the compounds whilst some derivatives showed potency higher than the standard used.

Introduction

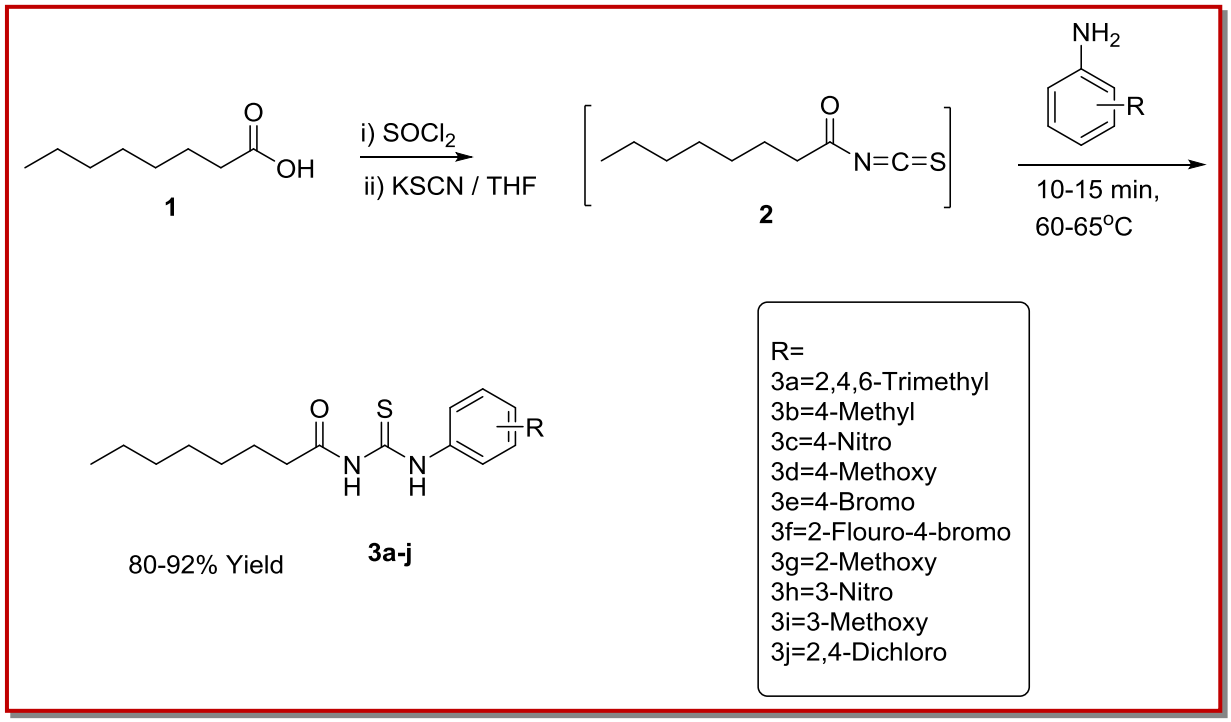
Thioureas find extensive utility in synthetic, biological and commercial fields. It possesses a broad spectrum of biological activities including antibacterial, antifungal, anti-oxidant, and enzyme inhibition. Thioureas are the key precursors for the synthesis of a wide variety of heterocycles. In recent decades, several new methods have also been reported for the preparation of substituted thioureas. A mild and efficient microwave assisted synthesis of various di- and tri-substituted thioureas have been reported. Our research group has extensively been involved in the synthesis, biological assay and synthesis of heterocycles from thioureas (Saeed et al., 2015; Saeed et al., 2015; Saeed et al., 2014 and Saeed et al., 2016) their biological activities and theoretical studies have comprehensively been published (Saeed et al., 2015; Saeed et al., 2015; Saeed et al., 2015; Saeed et al., 2014; Zaib et al., 2014; Saeed et al., 2013; Saeed et al., 2014; Saeed et al., 2010; Saeed et al., 2016; Saeed et al., 2009; Saeed et al., 2010 and Saeed et al., 2011). Thus, application of thioureas as excellent targets in medicinal chemistry has already been well established.

Herein, we report a clean, efficient and the solvent-free synthesis of a small library of 1-aroyl thioureas and their evaluation as antibacterial, antifungal, anti-oxidant and enzyme inhibitors.

Materials and Methods

Experimental

The mild solvent-free synthesis of a small library of ten candidates (3a-3j) was achieved by stirring freshly



Scheme 1: Synthesis of substituted aryl thioureas

prepared octanoyl isothiocyanate with suitably substituted anilines. The complete description of the substituents for derivatives is enunciated in Scheme 1. This method provides facile access towards the synthesis of thiourea derivatives by employing solvent free conditions in a very short time. Moreover, the yield obtained by employing this methodology is excellent.

The melting points were determined on a Bio Cote SMP10-UK and are uncorrected. The chemicals like octanoic acid, ammonium thiocyanate, thionyl chloride, and aromatic amines, were purchased from Sigma-Aldrich and are used as received**.** The experiments were carried out in standard pyrex capable glassware chamber (20 mL). NMR spectra were recorded on a Bruker ARX, 300 MHz spectrometer, ^{1}H NMR (300.13 MHz) and ^{13}C NMR (75.47 MHz) using internal standard $CDCl_3$ solutions (7.28 ppm from TMS). The splitting of proton resonances in the reported ^{1}H NMR spectra are defined as s singlet, d doublet, t triplet, q quartet and m complex pattern; coupling constants were reported in Hz. FT-IR spectra were recorded as KBr pellets on a Bio -Rad Excalibur FT-IR model FTS 3000 MX (400-4000 cm^{-1}) and the elemental analyses were performed using a LECO-932 CHNS analyzer.

Antibacterial assays

The antibacterial activity of the compounds was evaluated by disc diffusion assay as reported previously (Ellman et al., 1964). In experiment two Gram positive [*Staphylococcus aureus* (ATCC 6538) and *Micrococcus luteus* (ATCC 10240)] and two Gram negative [*Escherichia coli* (ATCC 15224) and *Enterobacter aerogens* (ATCC 13048)] were cultured in nutrient broth for 24 hours at 37°C. These cultured strains were used as inoculums (1%) to run the assay. Each bacterial strain was added to the nutrient agar medium at 45°C, poured into sterile petri plates and allowed to solidify. 5 µL of the test compound with a final concentration of 200 µg/mL was poured on sterile filter paper discs (4 mm) and placed on nutrient agar plates. Kanamycin and DMSO were used as positive- and-negative controls, respectively on each plate. The assay was performed in triplicate and the plates were incubated at 37°C for 24-48 hours. The antibacterial activity of the compounds was determined by measuring the diameter of zones showing complete inhibition (mm) with the help of Vernier caliper.

Antifungal assay

Antifungal activity of synthesized compounds was measured by previously reported disc diffusion method (Mohammed et al., 2011) against *Mucor species* (FCBP 0300), *Aspergillu sniger* (FCBP 0198), *Aspergillus flavus* (FCBP 0064) and *Fusarium solani* (FCBP 0291). All fungal strains were cultured on Sabouraud dextrose agar (SDA) at 28°C for 5-7 days. Actively growing fungal spores of each strain were spread with the help of autoclaved cotton swaps on solidified SDA petri plates under sterile conditions. 5 µL of each test compound with a final concentration of 200 µg/mL was poured on sterile filter paper discs (4 mm) and placed on SDA plates respectively. Terbinafine and DMSO served as positive and negative controls, respectively on each plate. All plates were incubated at 28°C for 5-7 days and

fungal growth was determined by measuring the growth diameter (mm) with the help of a Vernier caliper.

Anti-oxidant activity

Radical scavenging activity of test compounds against stable free radical 2,2 diphenyl-1-picryl-hydrazyl (DPPH) was determined spectrophotometrically. Each test compound (5 µL) with the final concentration of 200, 100 and 50 µg/mL was mixed with 100 µM DPPH (95 µL) in 96-well microtiter plates. Ascorbic acid and DMSO were used as positive- and negative-control respectively. The experiment was performed in triplicate and reaction mixtures were incubated in dark for 30 min at 37°C in dark. After incubation, absorbance was measured at 515 nm by using microplate reader (BioTeK, Elx 800). IC_{50} was calculated with Graph pad Prism 5.

α-Amylase assay

The compounds were tested for their enzyme inhibition activity against α-amylase by the previously reported method (Gorja et al., 2013). For assay 5 µL of each test compound with the final concentration of 200, 100 and 50 µg/mL was mixed with 40 µL of starch (0.05%) and 30 µL of potassium phosphate buffer (pH 6.8) in 96-well micro titer plates followed by the addition of 10 µL of α-amylase enzyme (0.2 U/well). Acarbose and DMSO were used as positive- and negative-control respectively. The plates were incubated for 30 min at 50°C and 20 µL HCl (1M) as stopping reagent was added. Then 100 µL of iodine reagent (5 mM KI and 5 mM I_2) was added to check the presence and absence of starch and absorbance was measured at 540 nm with microplate reader (Bio Tek, Elx800). The experiments were performed in triplicate and IC_{50} was calculated with Graph pad Prism 5.

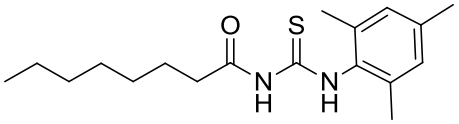
Butyrylcholinesterase assay

Ellman's method was used to determine the enzyme inhibition potential of the compounds against butyryl cholinestrase (BChE) (Ellman et al., 1966). In experiment butyrylthiocholine iodide (BChI) was used as substrates and assay was performed in triplicate in 96-well plates. The compound (5 µL) with final concentration of 200, 100 and 50 µg/mL was mixed with 20 µL of 100 µM sodium phosphate buffer (pH 8.0) and 5 µL BChE enzyme (0.05 U/mL). Then 10 µL BChI (4 mM) and 60 µL DTNB (3 mM) was added. Galantamine hydrobromide (Sigma) and DMSO served as positive- and negative-controls respectively. The reaction mixtures were then incubated for 30 min at 37°C. After incubation absorbance was measured at 405 nm using a microplate reader (Bio Tek Elx-800, USA) and IC_{50} was calculated by using Graph pad Prism 5.

General procedure

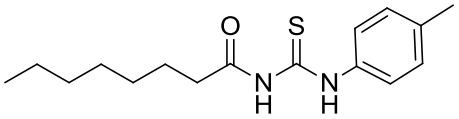
Freshly prepared octanoyl isothiocyanate was treated with respective aryl amines in a 1:1 Molar ratio under dry conditions. The reaction mixture was stirred at 60-65°C for about 10-15 min. On cooling, the reaction mixtures were slowly poured into acidified (pH 4-5) chilled water and stirred well (50 mL). The solid products obtained were separated by filtration and dried at room temperature.

***N*-(Mesitylcarbamothioyl)octanamide (3a)**



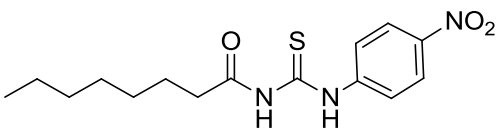
Light yellow solid; Yield: 91%; Rf (n-hexane: Ethyl acetate 1:1); 0.56; m.p: 195°C; IR (neat,cm^{-1}): 3219 (m, N-H), 1680 (s, v C=O), 1534 (v, d, NH), 1268 (v CNC). ^{1}H NMR (DMSO-d6), 300 MHz): 12.65 (s, 1H, NH), 12.03 (s, 1H, NH), 7.48 (d, 1H, *J* = 8.3, Ar-H), 3.21 (s, 6H, Ar-H), 2.80 (s, 2H, Ar-H), 2.42 (t, 2H, *J*=3Hz), 1.93 (quint, 2H, *J*=2Hz), 1.61 (quint, 2H), 1.32 (quint, 2H), 1.11 (quint, 2H), 0.90 (sex, 2H), 0.61 (t, 3H, *J*=1Hz) ;^{13}C NMR (DMSO-d_6);75.5 MHz): 179.6, 170.4, 162.19, 140.1, 133.4, 132.1, 130.3, 37.5, 32.3, 25.2, 22.1, 18.3, 15.4, 12.8, Anal. Calcd. For $C_{18}H_{28}N_2OS$, C, 67.25; H, 8.81; N, 8.71; S 10.1; Found: C, 66.19; H, 8.60; N, 8.32; S, 9.74.

***N*-(p-Tolylcarbamothioyl)octanamide (3b)**



Light Pink solid; Yield: 88%; Rf (n-hexane: Ethyl acetate 1:1); 0.50; m.p: 197°C; IR (neat,cm^{-1}): 3222 (m, N-H), 1688 (s, v C=O), 1540 (v, d, NH), 1275 (v CNC). ^{1}H NMR (DMSO-d_6), 300 MHz): 12.61 (s, 1H, NH), 11.89 (s, 1H, NH), 7.5 (dd, 2H, *J* = 8.3, *J*=8.0 Hz, Ar-H), 8.13 (dd, 2H, *J* = 7.0 Hz, J=6.7 Hz, Ar-H), 2.7 (s, 2H, Ar-H), 2.2 (t, 2H, *J*=3Hz), 1.8 (quint, 2H, *J*=2Hz), 1.5 (quint, 2H), 1.2 (quint, 2H), 1.0 (quint, 2H), 0.8 (sex, 2H),0.5 (t, 3H, *J*=1Hz); ^{13}C NMR (DMSO-d_6); 75.5 MHz): 178.6, 173.4, 163.19, 141.1, 134.4, 132.5, 44.3, 372, 321, 256, 22.7, 15.2, 12.4, 10.2; Anal. Calcd. For $C_{16}H_{24}N_2OS$, C, 65.71; H,8.27; N, 8.27; S 10.96; Found: C, 64.19; H, 7.8; N, 7.75; S, 9.74.

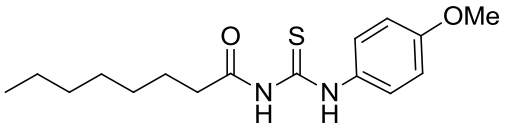
N-((4-Nitrophenyl)carbamothioyl)octanamide (3c)



Yellow solid; Yield: 80%; Rf (n-hexane: Ethyl acetate 1:1); 0.61; m.p: 183°C; IR (neat, cm^{-1}): 3229 (m, N-H), 1685 (s, v, C=O), 1540 (v, d, NH), 1279 (v CNC). ^{1}H NMR (DMSO-d_6), 300 MHz): 12.65 (s, 1H, NH), 12.03 (s, 1H, NH), 8.23 (d, 1H, *J* = 8.3, Ar-H), 7.83 (d, 1H, *J* = 8.03,

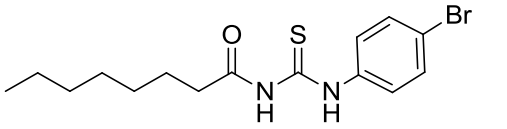
Ar-H), 2.5 (t, 2H, *J*=3Hz), 2.01 (quint, 2H, *J*=2Hz), 1.62 (quint, 2H), 1.42 (quint, 2H), 1.32 (quint,2H), 1.12 (sex, 2H), 0.81 (t, 3H, *J*=1Hz) ; ^{13}C NMR (DMSO-d_6); 75.5 MHz): 180.6, 175.4, 165.19, 142.1, 135.4, 133.1, 131.4, 43.4., 39.3, 36.4, 24.5, 16.2, 14.5, Anal. Calcd. For $C_{15}H_{21}N_3O_3S$, C, 55.71; H, 6.51; N, 12.71; S 9.91; Found: C, 54.19; H, 6.60; N, 11.32; S,8.74.

***N*-((4-Methoxyphenyl)carbamothioyl)octanamide (3d)**



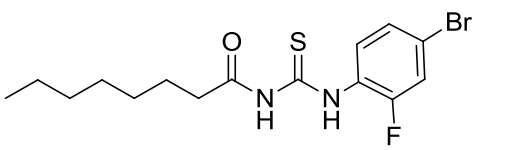
Light yellow solid; Yield: 95%; Rf (n-hexane: Ethyl acetate 1:1); 0.61; m.p: 192°C; IR (neat, cm^{-1}): 3234 (m, N-H), 1671 (s, v C=O), 1544 (v, d, NH), 1265 (v CNC). ^{1}H NMR (DMSO-d_6), 300 MHz): 12.31 (s, 1H, NH), 11.44 (s, 1H, NH), 7.83 (d, 1H, *J* = 8.12, Ar-H), 8.13 (d, 1H, *J* = 7.91, Ar-H), 3.98 (s, 3H), 2.42 (t, 2H, *J*=3Hz), 1.90 (quint, 2H, *J*=2Hz), 1.62 (quint, 2H), 1.32 (quint, 2H), 1.14 (quint, 2H), 0.9 (sex, 2H),0.62 (t, 3H, *J*=1Hz); ^{13}C NMR (DMSO-d_6); 75.5 MHz): 179.6, 170.4, 162.19, 140.1, 133.4, 132.1, 54.6, 45.4, 40.2, 32.2, 25.4, 18.6, 12.3, 10.7 Anal. Calcd. For $C_{16}H_{24}N_2O_2S$, C, 62.30; H,7.84; N, 9.08; S 10.40; Found: C, 62.19; H, 7.60; N, 8.32; S, 9.74.

N-((4-Bromophenyl)carbamothioyl)octanamide (3e)



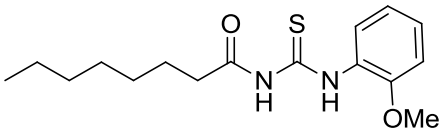
Yellow solid; Yield: 98%; Rf (n-hexane: Ethyl acetate 1:1); 0.51; m.p: 187°C; IR (neat, cm^{-1}): 3229 (m, N-H), 1685 (s, v, C=O), 1540 (v, d, NH), 1279 (v CNC). ^{1}H NMR (DMSO- d_6), 300 MHz): 12.65 (s, 1H, NH), 12.03 (s, 1H, NH), 7.63 (d, 1H, *J*= 8.3, Ar-H), 7.65 (d, 1H, *J* = 8.0, Ar-H), 2.4 (t, 2H, *J*=3Hz), 2.1 (quint, 2H, *J*=2Hz), 1.7 (quint, 2H), 1.5 (quint, 2H), 1.4 (quint, 2H), 1.2 (sex, 2H),0.9 (t, 3H, *J*=1Hz); ^{13}C NMR (DMSO-d_6); 75.5 MHz): 181.6, 174.4, 164.19, 143.1, 136.4, 134.1, 41.6, 37.2, 34.5, 30.4, 26.4, 23.7, 18.6, Anal. Calcd. For $C_{15}H_{21}BrN_2OS$, C, 50.71; H, 5.92; N, 7.84; S 8.96; Found: C, 49.19; H, 6.60; N, 8.32; S, 7.74.

***N*-((4-Bromo-2-fluorophenyl)carbamothioyl) octanamide (3f)**



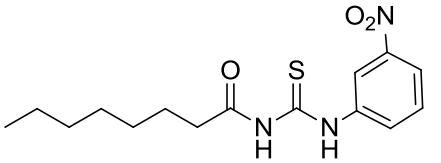
White solid; Yield: 86%; Rf (n-hexane: Ethyl acetate 1:1); 0.58; m.p: 201°C; IR (neat, cm^{-1}): 3230 (m, N-H), 1676 (s, v C=O), 1547 (v, d, NH), 1269 (v CNC). ^{1}H NMR (DMSO-d_6), 300 MHz): 12.11 (s, 1H, NH), 11.68 (s, 1H, NH), 7.93 (d, 1H, *J* = 7.10, Ar-H), 7.56 (d, 1H, *J* = 7.61, Ar -H), 2.60 (t, 2H, *J*=3Hz), 2.01 (quint, 2H, *J*=2Hz), 1.82 (quint, 2H), 1.40 (quint, 2H), 1.32 (quint, 2H), 1.12 (sex, 2H),0.61 (t, 3H, *J*=1Hz); ^{13}C NMR (DMSO-d_6); 75.5 MHz): 179.6, 170.4, 162.19, 140.1, 133.4, 132.1, 45.3, 40.5, 37.2, 32.4, 25.2, 22.4, 18.5, 15.5, 12.3, Anal. Calcd. For $C_{15}H_{20}BrFN_2OS$, C, 48.30; H, 5.37; N, 7.46; S 8.54; Found: C, 47.19; H, 7.50; N, 7.21; S, 9.74.

N-((2-Methoxyphenyl)carbamothioyl)octanamide (3g)



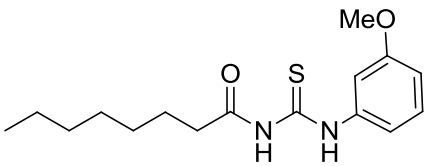
Black solid; Yield: 84%; Rf (n-hexane: Ethyl acetate 1:1); 0.63; m.p: 210°C; IR (neat, cm^{-1}): 3245 (m, N-H), 1679 (s, v C=O), 1549 (v, d, NH), 1269 (v CNC). ^{1}H NMR (DMSO-d_6), 300 MHz): 11.14 (s, 1H, NH), 11.68 (s, 1H, NH), 7.73 (d, 1H, *J* = 7.13, Ar-H), 7.56 (d, 1H, *J* = 7.45, Ar -H), 7.06 (d, 1H, *J* = 7.13, Ar-H), 7.07 (d, 1H, *J* = 7.45, Ar-H), 3.45 (s, 3H, OCH_3), 2.6 (t, 2H, *J*=4Hz), 2.0 (quint, 2H, *J*=3Hz), 1.8 (quint, 2H), 1.4 (quint, 2H), 1.3 (quint, 2H), 1.1 (sex, 2H),0.6 (t, 3H, *J*=1Hz) ;^{13}C NMR (DMSO-d_6); 75.5 MHz): 176.6, 171.4, 160.19, 141.1, 137.4, 135.4, 50,45, 41.6, 37.5, 32.4, 25, 22.3, 18.4, 15.8, 12.2, 14.1 Anal. Calcd. For $C_{16}H_{24}N_2O_2S$, C, 62.30; H, 7.84; N, 9.08; S, 10.40 Found: C, 47.19; H, 7.50; N, 7.21; S, 9.74.

***N*-((3-Nitrophenyl)carbamothioyl)octanamide (3h)**



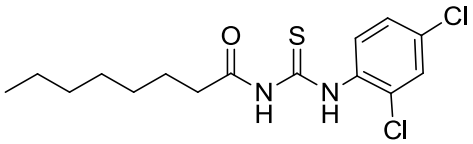
Yellow solid; Yield: 80%; Rf (n-hexane: Ethyl acetate 1:1); 0.53; m.p: 170°C; IR (neat, cm^{-1}): 3229 (m, N-H), 1670 (s, v C=O), 1543 (v, d, NH), 1260 (v, CNC). ^{1}H NMR (DMSO-d_6), 300 MHz): 12.22 (s, 1H, NH), 11.26 (s, 1H, NH), 7.93 (d, 1H, *J* = 7.10, Ar-H), 7.56 (d, 1H, *J* = 7.61, Ar-H), 7.92 (d, 1H, *J* = 7.10, Ar-H), 7.86 (d, 1H, *J* = 7.61, Ar-H), 2.6 (t, 2H, *J*=3Hz), 2.0 (quint, 2H, *J*=2Hz), 1.8 (quint, 2H), 1.4 (quint, 2H), 1.3 (quint,2H), 1.1 (sex, 2H),0.6 (t, 3H, *J*=1Hz); ^{13}C NMR (DMSO-d_6); 75.5 MHz): 179.6, 170.4, 162.19, 140.1, 133.4, 132.1, 46.4, 40.7, 37.3, 32.4., 25.8, 22.6, 18.6, 15.4, 12.8, Anal. Calcd. For $C_{15}H_2N_3O_3S$, C, 55.71; H, 6.54; N, 12.99; S 9.91; Found: C, 54.19; H, 6.40; N, 11.21; S, 8.74.

***N*-((3-Methoxyphenyl)carbamothioyl)octanamide (3i)**



Yellow solid; Yield: 88%; Rf (n-hexane: Ethyl acetate 1:1); 0.51; m.p: 187°C; IR (neat, cm^{-1}): 3226 (m, N-H), 1680 (s, v, C=O), 1535 (v, d, NH), 1270 (v CNC). ^1H NMR (DMSO-d_6), 300 MHz): 12.12 (s, 1H, NH), 11.76 (s, 1H, NH), 7.23 (dd, 1H, *J* = 8.3, *J*=7.2Hz, Ar-H), 7.83 (dd, 1H, *J* = 8.0, *J*=7.4Hz, Ar-H), 7.43 (dd, 1H, J=7.12 Hz), 7.41 (dd, 1H, J=7.12 Hz), 3.7 (s, 3H, OCH_3), 2.5 (t, 2H, *J*=4Hz), 2.0 (quint, 2H, *J*=3Hz), 1.6 (quint, 2H), 1.4 (quint, 2H), 1.3 (quint, 2H), 1.1 (sex, 2H), 0.8 (t, 3H, *J*=1Hz); ^{13}C NMR (DMSO-d_6 75.5 MHz): 179.21, 174.4, 165.19, 142.1, 135.4, 133.1, 56,43, 39.8, 36.9, 30.6, 24.8, 21.5, 16.8, 14.7, 12.8, 9.1; Anal. Calcd. For $C_{15}H_{21}N_3O_3S$, C, 55.71; H, 6.51; N, 12.71; S 9.91; Found: C, 54.19; H, 6.60; N, 11.32; S, 8.74.

N-((2,4-Dichlorophenyl)carbamothioyl)octanamide (3j)



White solid; Yield: 86%; Rf (n-hexane: Ethyl acetate 1:1); 0.49; m.p: 198°C; IR (neat, cm^{-1}): 3234 (m, N-H), 1670 (s, v C=O), 1549 (v, d, NH), 1261 (v CNC). ^1H NMR (DMSO-d_6), 300 MHz): 12.25 (s, 1H, NH), 11.63 (s, 1H, NH), 7.83 (d, 1H, *J* = 7.12, Ar-H), 7.53 (d, 1H, *J* = 7.52, Ar -H), 7.52 (d, 1H, *J* = 7.52, Ar-H), 2.8 (t, 2H, *J*=3Hz), 2.11 (quint, 2H, *J*=2Hz), 1.93 (quint, 2H), 1.32 (quint, 2H), 1.1 (quint,2H), 0.92 (sex, 2H),0.86 (t, 3H, *J*=1Hz) ;^{13}C NMR (DMSO-d_6); 75.5 MHz): 178.6, 173.4, 164.19, 142.1, 135.2, 131.7, 42.9, 39.5, 35.7, 33.9, 27.8, 24.9, 19.7, 16.5, 14.9, Anal. Calcd. For $C_{15}H_{20}BrFN_2OS$, C, 51.30; H, 5.80; N, 8.06; S 9.24; Found: C, 50.19; H, 5.50; N, 7.21; S, 8.74.

Results

Antibacterial assay

The synthesized compounds were evaluated for their potential antibacterial activity by using four different bacterial strains (Table I). The results showed that two compounds **3a** and **3c** showed antibacterial activity against all the tested bacterial strains which represent that these compounds are equally activity against Gram positive as well as Gram negative bacteria. The compound **3i** exhibited antibacterial good activity against three bacterial strains except *Enterobacter aerogenes*. The compounds **3g** and **3h** showed antibacterial activity only against *Staphylococcus aureus*. Overall a range of antibacterial activity has been exhibited by the tested compounds.

Antifungal assay

All the tested compounds (**3a**-**3j**) showed significant antifungal activity against all tested fungal strains indicating that these compounds are broad spectrum antifungal candidates (Table II). The highest activity was measured against *Aspergillus flavus* and *Aspergillus niger*. These are very interesting findings that some compounds, e.g **3a** and **3c** possess both antibacterial as well as significant antifungal activity against all tested strains.

Butyryl cholinesterase assay and anti-oxidant assay

The compounds were screened for their radical scavenging activity through DPPH assay (Table III). The results showed that **3a**, **3b**, **3c** and **3d** were found anti-oxidant and the rest of the compounds did not show any anti-oxidant activity.

a-Amylase assay

The compounds were evaluated for their enzyme inhibition potential against α-amylase enzyme and results in the form of IC_{50} values are given in Table III. Experiment was performed in triplicates and acarbose (IC_{50} 17.1 µg/mL) was used as positive control. The results showed that the compounds **3f**, **3g** and **3h** exhibited enzyme inhibition activity with IC_{50} values of 282.1, 294.2 and 285.1 µg/mL respectively.

Table I

Antibacterial activity of the compounds

SL. No.	Compound code	Zones of inhibition (mm) at 200 µg/mL			
		Micrococcus luteus	*Staphylococcus aureus*	*Escherichia coli*	*Enterobacter aerogenes*
1	3a	25	27	26	25
2	3b	27	28	30	25
3	3c	30	32	29	26
4	3d	29	28	30	22
5	3e	27	19	22	19
6	3f	25	17	24	20
7	3g	20	16	26	20
8	3h	23	17	20	10
9	3i	18	18	16	20
10	3j	12	13	17	15
11	Kanamycin	32	34	28	24

Table II

Antifungal activity of the compounds

SL. No.	Compound code	Zones of inhibition (mm) at 200 µg/mL			
		Fusarium solani	*Mucor species*	*Aspergillus flavus*	*Aspergillus niger*
1	3a	17	18	14	10
2	3b	6	17	10	12
3	3c	8	10	12	13
4	3d	18	11	16	10
5	3e	25	15	18	26
6	3f	15	25	15	28
7	3g	25	16	27	15
8	3h	5	15	15	10
9	3i	16	18	20	18
10	3j	26	18	20	25
11	Terbinafine	24	22	28	24

Table III

IC_{50} values (µg/mL) of DPPH, α-amylase and butyrylcholineasterase assay

SL. No.	Compound code	IC_{50} values (µg/mL)		
		DPPH	α-amylase	butyrylcholineasterase
1	3a	12	22	-
2	3b	10	25	185.8
3	3c	13	12	-
4	3d	10	9	-
5	3e	-	-	-
6	3f	246.3	282.1	238.8
7	3g	-	294.2	-
8	3h	-	-	-
9	3i	-	285.1	271.4
10	3j	151.2	-	-
11	Ascorbic acid	11.9	-	-
12	Acarbose	-	17.1	-
13	Galantaminehydrobromide	-	-	4.6

Butyryl cholinesterase assay

The synthesized compounds were screened for their inhibition ability against butyryl cholinesterase enzyme. The experiment was performed in triplicates and galantamine hydrobromide was used as a positive control (IC_{50} 4.6 µg/mL). It can be seen from results that three compounds **3b** (IC_{50} 185.8 µg/mL), **3f** (IC_{50} 238.8 µg/mL) and **3i** (IC_{50} 271.4 µg/mL) were found potential inhibitor of butyrylcholinesterase enzyme while the rest of the compounds were not active against enzyme (Table III).

Table III shows the results of anti-oxidant activity and enzyme inhibition. The compounds were subjected for enzyme inhibition for two different enzymes.

Discussion

Previously, the reported synthesis of thioureas by conventional method has not delivered excellent yield and tedious reaction work ups were involved and the reaction was completed in 5 hours.

It is well recognized that thioureas possess a wide spectrum of biological activities and previously our research group and other researchers have highlighted the biological application of different acyl and aryl thioureas. To the best our knowledge, there is no paper published related to the octanoyl thiourea. However, Correa et al. (2015) reported the BSA- and DNA-binding studies of thiourea complexes against lung and prostate tumor cells. The antimicrobial activities of thioureas were reported in which few derivatives were found to be good against *E. coli* (Zhong et al., 2008). Madabhushi et al. (2014) reported the benzimidazole linked chiral thioureas as antibacterial and anti-cancer agents. The biological applications of acyl/aryl thioureas have comprehensively been discussed (Saeed et al., 2014). Tameryn et al. published the antiparasitic

and cytotoxic activities of polyamide thioureas (Stringer et al., 2013). Saeed et al. reported the coumarin linked thioureas as cholinesterase inhibitors (Saeed et al., 2015). Saeed et al reported amino benzene sulfonamide thiourea conjugates as carbonic anhydrase inhibitors (Saeed et al., 2014). But herein, we have reported the multi-target activities of synthesized thioureas.

But all these other reported work on thioureas have demonstrated their different biological activities, herein, we first time, report the multi-target potential of octanoyl thioureas.

The nature of substrates H_2NR, strongly influence the yield of the final products obtained (Scheme 1). The substituents like F, Br, Cl, methyl, OMe, attached at the ortho, meta and para positions on the aromatic ring in H_2NR moiety show the strong mesomeric effect by releasing electrons through delocalization of lone pairs in spite of the inductive effect (-I) that result in an increase of nucleophilic character of the amino group. It is well established that the resonance effect is stronger than inductive effect and the net result is electron releasing to rest of the molecule. However, the reactivity of di- and higher substituted substrate has been enhanced owing to their increased electron releasing capability and of better yield as the data suggest. This has been reflected in the increase of product yield in the following order; Br > methyl > OM > F. The nitro-containing substrates have shown lowest yield due to its electron pulling nature from the aromatic ring, rendering the ring electron deficient, which in turn diminishes the nucleophilic character of amine.

The significant absorptions observed in the FT-IR spectra of all the synthesized substituted thioureas are listed in spectroscopic data along with the respective compounds in the experimental section. The tentative assignments for functionalities are made according to the literature (Saeed et al., 2014). The absence of υ (S-H) vibration in the range of 2529-2588 cm^{-1} confirmed the conversion of isothiocyanate moiety into thiourea (-NHCSNH-) functionality. The substituted thioureas behave both as a monodentate and bidentate ligands, depending upon the reaction conditions. The characteristic IR bands of substituted thioureas are found around; 3120-3402 (NH), 2960-3090 Ph(CH), 1660-1720 (C=O), 1540-1620 (CN), 1243-1277 (C=S) and 1130-1185 (C-S) (Saeed et al., 2013). The addition of the amine to the C-N double bond took place by attacking the carbon atom in the isothiocyanate group resulting in the formation of the desired compounds. After complete reaction, the strong band at 2000 cm^{-1} (N-C-S) in the isothiocyanate disappeared (Saeed et al., 2016). Instead of the expected normal carbonyl (C-O) absorption around 1710 cm^{-1} a medium strong band at 1665-78 cm^{-1} suggested a possible hydrogen bond formation between the H-atom of the NH-group and the O-atom of the carbonyl group. The effect and type of hydrogen bonding in thioureas have been excellently discussed by us in earlier reported paper [Saeed et al., 2011]. The ^1H NMR data for the synthesized disubstituted thioureas showed that the NH hydrogen resonates considerably down-field from other resonances in the spectra. The proton chemical shifts were found around 11-12 ppm for free and hydrogen bonded NH, respectively, and aromatic protons appeared down-field between 7.23 and 8.50 ppm in their usual regions. It was also observed in our previous work that the coordinating or highly polar solvents like DMSO-d_6 had a profound effect on the free NH protons chemical shift and appeared more downfield as compared with the non-coordinating solvents like $CDCl_3$, C_6D_6 and CD_2Cl_2. This shift could be attributed to the possible hydrogen bonding between the NH and sulfoxide (S-O) moiety (Saeed et al., 2014). The ^{13}C NMR data explicitly show all the signals due to the distinct carbons present in compounds (**3a-i**). The aromatic carbon resonances of the thiourea 1 were assigned on the basis of signal intensities and then were compared with the reported values (Saeed et al., 2012). The chemical shifts of the carbons pertaining to CONH and CSNH moieties of the substituted thiourea ligands resonate around 166-68 and 177-80 ppm respectively.

The structure activity relationship was developed to rationalize the results of biological activities. The extensive applications of thiourea in biological field have prompted researchers to explore the structure-activity relationship. Thioureas are capable of showing inter and intra molecular hydrogen bonding which helps in acting as receptors as shown in Figure 1. A series of ten compounds were designed and synthesized to develop structure activity relationship for different five bioassays (antibacterial, antifungal, anti-oxidant and enzyme inhibition (α-amylase and butyl cholinesterase). Compounds **3a-d** showed better results in anti-oxidant activity, probably due to the substitution at para-position of the benzene ring. Compounds **3a-c** was found potent inhibitors against alpha-amylase enzyme and the series of compounds were found to be less potent against butylcholinesterase enzyme inhibition. In case of antibacterial and antifungal activity, the derivatives **3a-e** showed excellent results and **3c** derivative showed higher activity than the standard drug. Compounds **3a-e** with para-substitution showed better activity than meta and ortho substituted derivatives.

Conclusion

A series of new 1-octanoyl-3-aroyl was designed and synthesized. The biological assay results indicated that most of the compounds possessed *in vitro* antifungal activity against fluconazole-resistant *Trichophyton*

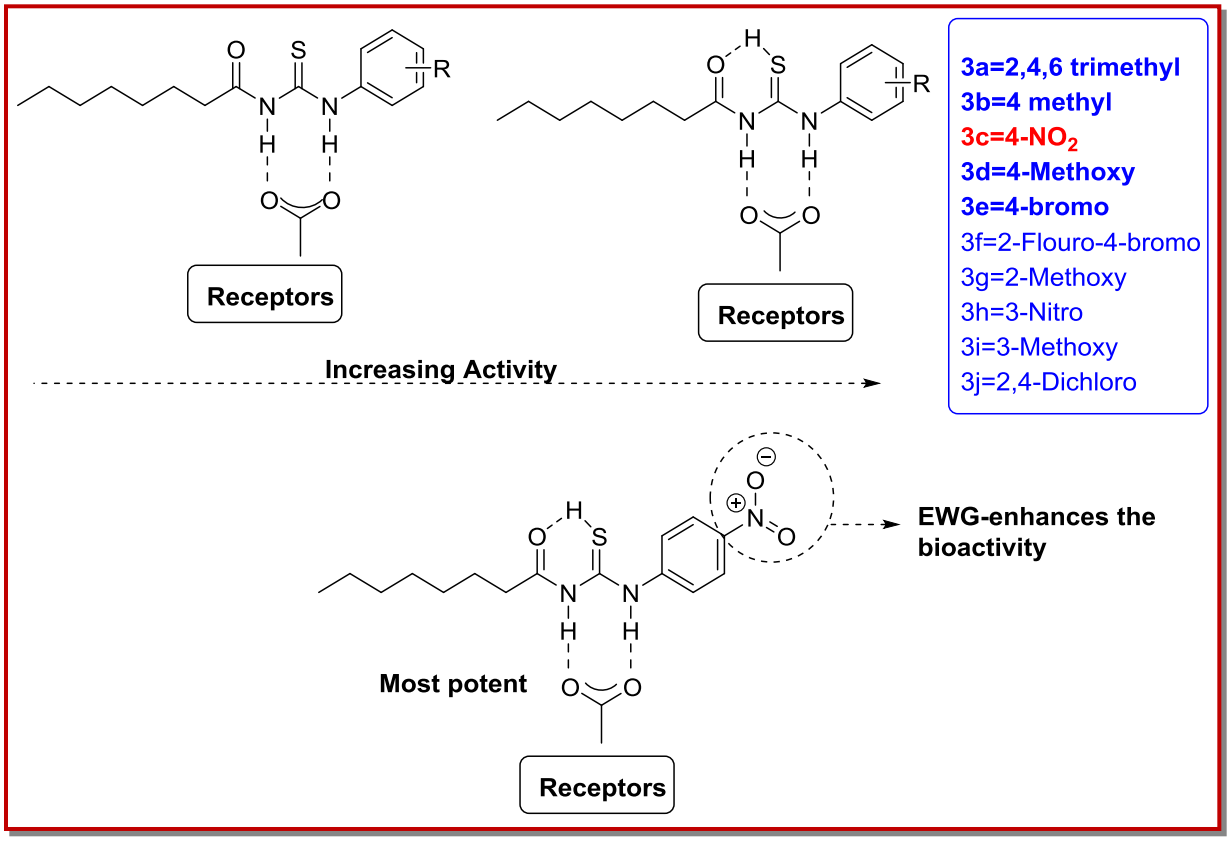


Figure 1: SAR relationship of series of 1-octanoyl-3-aroyl thioureas

rubrum and *Cryptococcus neoformans*. Compounds (**3a-i**) showed adequate activity.

Acknowledgement

We are thankful to Quaid-i-Azam University, Islamabad, Pakistan for providing a healthy environment for research.

Conflict of Interest

All authors have completed the ICMJE uniform disclosure form and declare no support from any organization for the submitted work.

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