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Synthesis, characterization and antibacterial evaluation of novel 2-mercaptobenzothiazole derivatives bearing 2-aminonicotinonitrile moiety

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ABSTRACT

An efficient methods has been described for the synthesis of new 2-aminonicotinonitrile derivatives (6a-f) bearing benzothiazole nucleus by solvent free microwave or ultrasound irradiation. The reaction proceeds via one-pot four-component by cyclo condensation reaction of heterocyclic methyl ketones (4a), malononitrile, ammonium acetate and aryl aldehydes. Chalcones intermediate and final compounds were also synthesized by conventional methods, the result confirms that microwave method showed higher yield and purity than conventional one. The synthesized compounds were characterized by FT-IR spectroscopy and ^{1}H NMR. Antibacterial activities of the synthesized compounds have been tested against four different types of bacteria; most of the tested compounds showed significant biological activities.

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1. Introduction

Microwave-assisted organic synthesis results in spectacular acceleration of many chemical reactions as a consequence of three-dimensional heating of the reaction mass, which cannot be reproduced by classical heating methods. High yields, improved selectivity, and clean reaction pathways are additional advantages of this synthetic technique [1]. Moreover, even reactions that do not occur with conventional heating can be performed with microwave irradiation. In most cases, microwave irradiation coupled with solvent-free techniques represents a powerful, eco-friendly, green alternative to conventional synthesis [2].

2-Mercaptobenzothiazole derivatives are known to possess various biological activities such as anti-inflammatory [3,4], anti-microbial [5-9], anticancer [10], anthelmintic activity [11], carbonic anhydrase inhibitor, treatment of gastroesophageal reflux disease and antihypertensive effect [12].

The *N*-aryl-3-substituted-5-pyrazolone derivatives are very useful intermediates in the synthesis of biologically active compounds exhibits widespread pharmacological properties such as: antibacterial, antifungal [13] anti-inflammatory [14], antioxidant [15], anticancer [16] and antitubercular activities [17].

A literature survey revealed that 2-aminonicotinonitrile (2-amino-3-cyanopyridine) nucleus derivatives belonging to an important group of heterocyclic compounds that have been the subject of extensive study in the recent past. It possesses diverse biological activities, such as: antibacterial, antifungal [18], anticancer activity [19], antituberculosis [20], anti-inflammatory and analgesic activities [21].

The development of new and different antibiotics provided the means for treating resistant strains of organisms that previously had been susceptible to an older antibiotic [22], these widespread applications and biological significance of 2-mercaptobenzothiazole, pyrazolone and aminonicotinonitrile containing compounds and in spite of the introduction of a variety of antibacterial agents in multiple unrelated drug classes, resistance continues to emerge. The pharmaceutical field must respond to these clinical challenges by bringing forward stream of new agents with promising antibacterial activity against bacteria [23].

Our goal is to synthesis new analogues of 2-aminonicotino nitrile and preliminary pharmacological evaluated as an antibacterial agents.

2. Experimental

2.1. Reagents and chemistries

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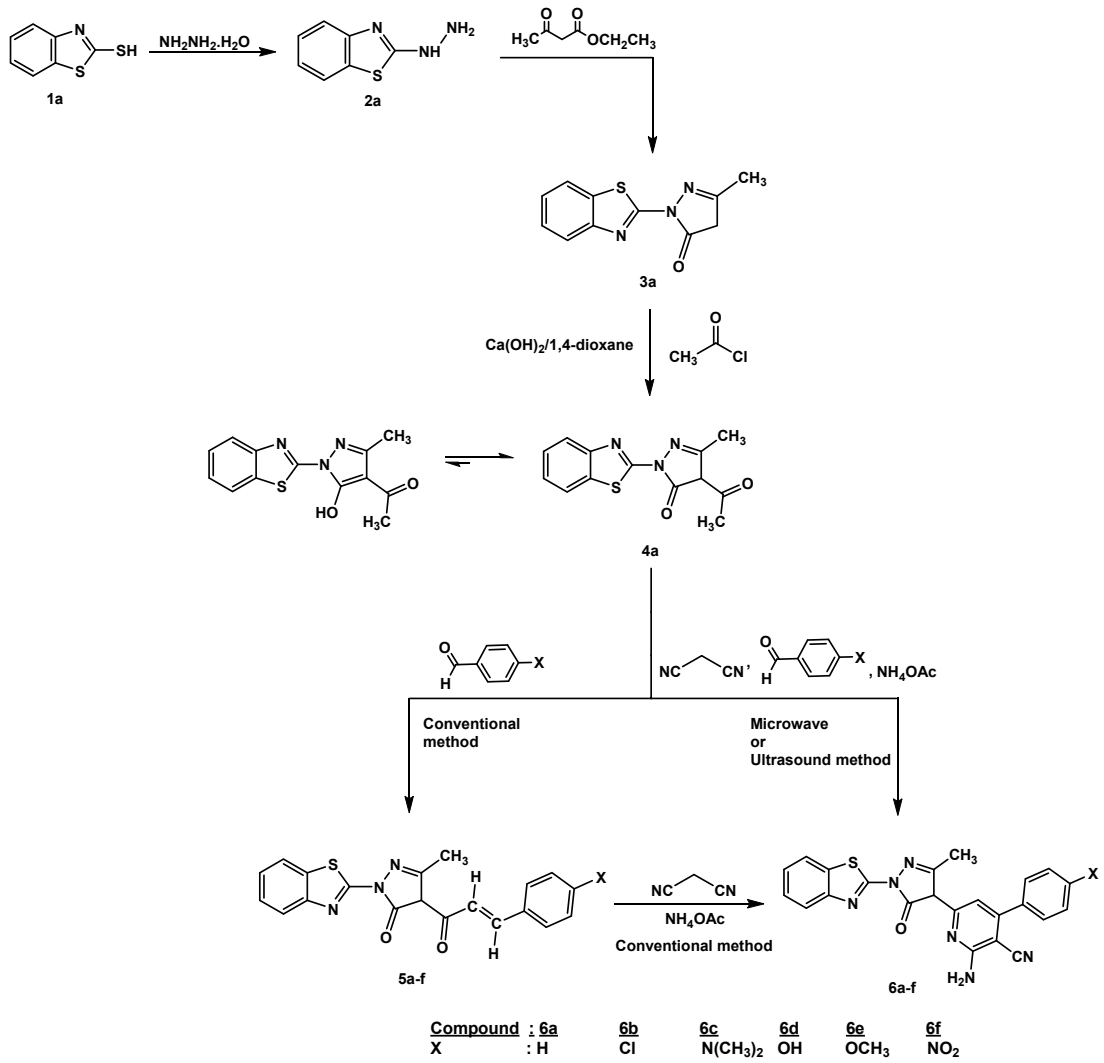

Scheme 1

All reagents and solvents were of analytical grade. All solvents were freshly distilled under anhydrous conditions. Microwave reactor (Anton Paar, USA) and Ultrasonic (ELMA) were used as reactor. Melting points were determined by open capillary method on Stuart/Electrothermal an electric melting point apparatus (U.K.) and ascending thin layer chromatography (TLC) to check the purity and progress of reactions was run on silica gel (60) $F_{254,}$ Merck (Germany). The identification of compounds was done using a U.V. detector and the chromatograms were eluted with ethylacetate: chloroform (4:6, *v*:*v*). IR spectra were recorded on a FT-IR spectrophotometer Shimadzu as KBr disks. ^{1}H NMR spectra were recorded using DMSO-d_6 as solvent and TMS as internal standard on a Bruker (400 MHz) spectrometer (Chemical shifts represented in δ, ppm). The general routes outlined in Scheme 1 were used to synthesize all compounds.

2.2. General procedure for synthesis of 2-hydrazinylbenzo [d]thiazole (2a)

2.2.1. Microwave method

2-Hydrazinylbenzo[*d*]thiazole (**2a**) was prepared by microwave method as per the reported earlier [8].

2.2.2. Ultrasound method

2-Hydrazinylbenzo[*d*]thiazole (**2a**) was prepared by ultrasound methods as per the reported earlier [24].

2.3. General procedure for synthesis of 1-(benzo[d]thiazol-2-yl)-3-methyl-1H-pyrazol-5(4H)-one (3a)

2.3.1. Conventional method

Ethyl acetoacetate (0.005 mol, 0.656 g) was added dropwise to 2-hydrazinobenzothiazole (**2a**) (0.005 mol, 0.825 g) in round bottom flask with constant stirring at room temperature for 15 min. A precipitate formed quickly. The reaction mixture was poured on crushed ice. The solid was separated, filtered and dried. Compound **3a** was recrystallized from ethanol [25]. Color: Deep orange to yellow. Yield: 83%. M.p.: 150-152 °C. FT-IR (KBr, ν, cm^{-1}): 3078 ν(CH, aromatic), 2964 ν(CH, aliphatic), 1737 ν(C=O, pyrazolone), 1649 ν(C=N), 1614 ν(C=C, aromatic). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.95 (s, 3H, CH_3), 3.45 (s, 2H, CH_2), 7.28-7.32 (t, 2H, Ar-H), 7.37-7.41 (d, 1H, Ar-H), 7.60 (d, 1H, Ar-H).

2.3.2. Microwave method

Ethyl acetoacetate (0.005 mol, 0.656 g) was added dropwise to corresponding 2-hydrazinobenzothiazole (**2a**) (0.005 mol, 0.825 g), the mixture was irradiated with microwave (300 W) for 4 min. The cold reaction mixture was treated with ethanol. The solid product was filtered, dried (the solid obtained was recrystallized from ethanol) [26]. Yield: 85%.

2.3.3. Ultrasound method

A solution of 2-hydrazinobenzothiazole (**2a**) (0.005 mol, 0.825 g) in ethanol (1 mL) was added dropwise to the corresponding ethyl acetoacetate (0.005 mol, 0.656 g) contained in a 25 mL conical flask. The mixture was irradiated in the water bath of an ultrasonic cleaner for 25 min. The cold reaction mixture was treated with ethanol. The solid product was filtered and dried. The solid obtained was recrystallized from ethanol [26]. Yield: 68%.

2.4. Synthesis of 4-acetyl-1-(benzo[d]thiazol-2-yl)-3-methyl-1H-pyrazol-5(4H)-one (4a)

The acylation reaction of compound **3a** was prepared as the reported method [27] and purified by recrystallization. *Keto-form* (Major tautomer): Color: Colourless. Yield: 75%. M.p.: 161-163 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.78-7.83 (d, 2H, Ar-H), 7.60-7.71 (t, 2H, Ar-H), 3.73 (s, 1H, CH), 2.93 (s, 3H, CH_3), 1.95 (s, 3H, CH_3). FT-IR (KBr, ν, cm^{-1}): 3236 ν(broad, OH), 3063 ν(C-H, aromatic), 2956, 2926 ν(C-H, aliphatic), 1748 ν(C=O, ketone), 1727 ν(C=O, pyrazolone), 1637 ν(C=N), 1602 ν(C=C, aromatic), 1365 ν(C-CH_3). *Enol-form* (Minor tautomer): Color: Yellow. Yield: 25%. M.p.: 173-176 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.7 (s, 1H, OH), 7.36-7.68 (d, 2H, Ar-H), 7.25-7.32 (t, 2H, Ar-H) , 3.55 (s, 3H, CH_3), 1.96 (s, 3H, CH_3).

2.5. Synthesis (E)-1-(benzo[d]thiazol-2-yl)-4-(3-(4-(substituted)phenyl)acryloyl)-3-methyl-1H-pyrazol-5(4H)-one as chalcone derivatives (5a-f)

4-Acetyl-1-(benzo[*d*]thiazol-2-yl)-3-methyl-1*H*-pyrazol-5(4*H*)-one (**4a**) (0.0037 mol, 1 g) was dissolved in 10 mL of ethanol in a round bottomed flask followed by (0.0037 mol) of *p*-substituted aromatic aldehyde and 4 drops of piperidine were added dropwise within 5 min. The mixture heated for 6 hours at 65 °C. The reaction mixture was cooled; the solid product was collected by filtration, washed with water and recrystallized from ethanol [28].

(E)-1-(1-(benzo[d]thiazol-2-yl)-5-hydroxy-3-methyl-1H-pyrazol-4-yl)-3-phenylprop-2-en-1-one (**5a**): Color: Deep brown. Yield: 65%. M.p.: 89-91 °C. FT-IR (KBr, ν, cm^{-1}): 3406 ν(OH), 3059 ν(Ar-H), 2918 ν(aliphatic, CH), 1705 ν(C=O, pyrazolone), 1695 ν(α,β-unsaturated ketone), 1633 ν(C=N), 1616 ,1518 ν(C=C, Ar). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.56 (s, 3H, CH_3), 6.79 (d, 1H, -CH=), 6.89 (d, 1H, =CH-Ar), 7.40-7.96 (m, 7H, Ar-H), 8.28 (d, 2H, Ar-H), 9.60 (s, 1H, OH).

(E)-1-(Benzo[d]thiazol-2-yl)-4-(3-(4-chlorophenyl)acryloyl)-3-methyl-1H-pyrazol-5(4H)-one (**5b**): Color: Deep gray. Yield: 67%. M.p.: 91-93 °C. FT-IR (KBr, ν, cm^{-1}): 3482 ν(OH), 3037 ν(ArH), 2960 ν(aliphatic, CH), 1710 ν(C=O, pyrazolone), 1696 ν(α,β-unsaturated ketone), 1619 ν(C=N), 1599, 1590 ν(C=C, Ar), 752 ν(C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.89 (s, 3H, CH_3), 3.30 (s, 1H, CH pyrazolone), 6.76 (d, 1H, -CH=), 7.48 (d, 1H, =CH-Ar), 7.48-7.52 (d, 2H, Ar-H), 7.59-7.63 (d, 2H, Ar-H), 7.69-7.78 (m, 2H, Ar-H) , 8.29 (d, 2H, Ar-H).

(E)-1-(1-(benzo[d]thiazol-2-yl)-5-hydroxy-3-methyl-1H-pyrazol-4-yl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one (**5c**): Color: Red. Yield: 80%. M.p.: 249-252 °C. FT-IR (KBr, ν, cm^{-1}): 3320 ν(OH), 3061 ν(ArH), 2912, 2821 ν(aliphatic, CH), 1715 ν(C=O, pyrazolone), 1677 ν(α,β-unsaturated ketone), 1662 ν(C=N), 1600, 1550 ν(C=C, Ar), 1375 ν(N-CH_3). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.64 (s, 3H, CH_3), 3.03 (s, 6H, -N(CH_3)$_2$), 6.06 (d, 1H, -CH=), 6.79 (d, 2H, Ar-H), 7.15 (d, 1H, =CH-Ar), 7.20-7.32 (d, 2H, Ar-H), 7.42(d, 2H, Ar-H), 8.08-8.18 (d, 2H, Ar-H), 10.07 (s, 1H, OH).

(E)-1-(1-(benzo[d]thiazol-2-yl)-5-hydroxy-3-methyl-1H-pyrazol-4-yl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**5d**): Color: Deep gray. Yield: 70%. M.p.: 97-100 °C. FT-IR (KBr, ν, cm^{-1}): 3416 ν(OH), 3063 ν(ArH), 2953 ν(aliphatic, CH), 1714 ν(C=O, pyrazolone), 1688 ν(α,β-unsaturated ketone), 1612 ν(C=N), 1588, 1515 ν(C=C, Ar), 1246 ν(C-O, phenolic). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.61 (s, 3H, CH_3), 6.62 (d, 2H, Ar-H), 6.89 (d, 1H,-CH=), 7.38 (d, 1H, =CH-Ar), 7.47-7.53(d, 2H, ArH), 7.59-7.64 (d, 2H, Ar-H), 7.80-7.82 (d, 2H, Ar-H), 9.55 (s, 1H, OH).

(E)-1-(Benzo[d]thiazol-2-yl)-4-(3-(4-methoxyphenyl) acryl oyl)-3-methyl-1H-pyrazol- 5(4H)-one (**5e**): Color: Brown. Yield: 69%. M.p.: 84-86 °C. FT-IR (KBr, ν, cm^{-1}): 3072 ν(ArH), 2960 ν(aliphatic, CH), 1710 ν(C=O, pyrazolone), 1690 ν(α,β-unsaturated ketone), 1633 ν(C=N), 1600, 1577 ν(C=C, aromatic), 1249 ν(OCH_3). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.55 (s, 3H, CH_3), 3.69 (s, 1H, CH pyrazolone), 3.81 (s, 3H, OCH_3), 6.70 (d, 1H, -CH=) 6.89 (m, 2H, Ar-H), 7.14 (d, 1H, =CH-Ar), 7.33-7.58 (d, 2H, ArH), 7.65-7.84 (m, 4H, Ar-H).

(E)-1-(Benzo[d]thiazol-2-yl) -3-methyl-4-(3-(4-nitrophenyl) acryloyl)-1H-pyrazol-5(4H)-one (**5f**): Color: Deep yellow. Yield: 75%. M.p.: 182-184 °C (Dec.). FT-IR (KBr, ν, cm^{-1}): 3369 ν(OH), 3063 ν(ArH), 2931 ν(aliphatic, CH), 1705 ν(C=O, pyrazolone), 1679 ν(α,β-unsaturated ketone), 1633 ν(C=N), 1600, 1519 ν(C=C, aromatic), 1489, 1344 ν(NO_2). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.53-1.59 (s, 3H, CH_3), 3.35-3.39 (s, 1H, CH of pyrazolone), 7.18 (d, 1H, -CH=), 7.57-7.85 (m, 4H, Ar-H), 8.13-8.18 (d, 1H, =CH-Ar), 8.37-8.41 (d, 2H, Ar-H), 8.87 (d, 2H, Ar-H).

2.6. Synthesis of 2-amino-6-(1-(benzo[d]thiazol-2-yl)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)-4-((4-subsituted)phenyl) nicotinonitrile as aminonicotinonitrile (6a-f)

2.6.1. Conventional method

To a solution of chalcone (**5a-f**) (0.01 mol) in 5 mL ethanol, (0.01 mol, 0.12 g) malononitrile and (0.03 mol, 0.398 g) ammonium acetate were added. Reaction mixture was refluxed at 60 °C for 8 hours, poured into crushed ice water, the product was extracted with 20 mL ethyl acetate. The organic layer was washed with brine, dry over sodium sulphate and evaporated under reduced pressure [29].

2.6.2. Ultrasound method

A mixture of equimolar (0.00075 mol) of malononitrile, substituted aromatic aldehydes and 4-acetyl-1-(benzo[*d*]thiazol-2-yl)-3-methyl-1*H*-pyrazol-5(4*H*)-one (**4a**) ammonium acetate and water (5 mL). The reaction mixture irradiated in ultrasonic bath for 18-30 min at 50 °C. The reaction was monitored by TLC. Reaction mixture was diluted with water (10 mL), filtered, washed with water and dried to give crude product, and recrystallized from ethanol [30].

2.6.3. Microwave method

A mixture of equimolar (0.00075 mol) of 4-acetyl-1-(benzo[*d*]thiazol-2-yl)-3-methyl-1*H*-pyrazol-5(4*H*)-one (**4a**) substituted aromatic aldehydes, malononitrile and ammonium acetate (0.0011 mol, 0.085 g) was irradiated by the microwave (7-9 min, 50 °C, 300 W). Reaction mixture was washed with ethanol. The crude products were recrystallized from ethanol [31].

2-Amino-6-(1-(benzo[d]thiazol-2-yl)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)-4-phenylnicotinonitrile (**6a**): Color: Dark brown. Yield: 62% (A), 41% (B), 68% (C). M.p.: 160-163 °C. FT-IR (KBr, ν, cm^{-1}): 3331, 3310 ν(NH_2), 3059 ν(ArH), 2926 ν(aliphatic, CH), 2191 ν(CN), 1712 ν(C=O, pyrazolone), 1631 ν(C=N). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.08 (s, 3H, CH_3), 3.84-3.89 (s, 1H, -CH for methine), 6.98 (s, 1H, CH, 2-Pyridine), 7.30-7.72 (m, 7H, Ar-H), 7.80-7.99 (d, 2H, Ar-H), 8.405 (broad s, 2H, NH_2).

2-Amino-6-(1-(benzo[d]thiazol-2-yl)-3-methyl-5-oxo-4, 5-dihydro-1H-pyrazol-4-yl)-4-(4-chlorophenyl) nicotinonitrile (**6b**): Color: Redish brown. Yield: 66% (A), 43% (B), 69% (C). M.p.: 107-108 °C. FT-IR (KBr, ν, cm^{-1}): 3336, 3279 ν(NH_2), 3059 ν(ArH), 2974 ν(aliphatic, CH), 2171 ν(CN), 1714 ν(C=O, pyrazolone), 1629 ν(C=N), 1055 ν(C-Cl). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.11(s, 3H, CH_3), 3.50-3.53 (s, 1H, -C-H for methine), 7.13 (s, 1H, CH, 2-Pyridine), 7.46-7.53 (d, 2H, Ar-H), 7.55-7.68 (m, 4H, Ar-H), 7.84 (d, 2H, Ar-H), 8.671 (broad s, 2H, NH_2).

2-Amino-6-(1-(benzo[d]thiazol-2-yl)-3-methyl-5-oxo-4, 5-dihydro-1H-pyrazol-4-yl)-4-(4-(dimethylamino)phenyl) nicotine nitrile (**6c**): Color: Yellow. Yield: 78% (A), 54% (B), 85% (C). M.p.: 128-130 °C. FT-IR (KBr, ν, cm^{-1}): 3317, 3232 ν(NH_2), 3082 ν(ArH), 2947 ν(aliphatic, CH), 2210 ν(CN), 1707 ν(C=O, pyrazolone), 1620 ν(C=N), 1371 ν(N-CH_3). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.84 (s, 3H, CH_3), 3.11 (s, 6H, -N(CH_3)$_2$), 3.76 (s, 1H, CH for methine), 6.63 (d, 2H, Ar-H), 7.09 (s, 1H, -CH for 2-Pyridine), 7.39 (d, 2H, Ar-H), 7.67-8.12 (m, 4H, Ar-H), 8.671 (broad s, 2H, NH_2).

2-Amino-6-(1-(benzo[d]thiazol-2-yl)-3-methyl-5-oxo-4, 5-dihydro-1H-pyrazol-4-yl)-4-(4-hydroxyphenyl) nicotinonitrile (**6d**): Color: Deep brown. Yield: 67% (A), 46% (B), 73% (C). M.p.: 138-140 °C. FT-IR (KBr, ν, cm^{-1}): 3373, 3295 ν(NH_2), 3061 ν(ArH), 2949 ν(aliphatic, CH), 2216 ν(CN), 1709 ν(C=O, pyrazolone), 1614 ν(C=N). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.22 (s, 3H, CH_3), 3.71 (s, 1H, CH methine), 6.61-6.79 (d, 2H, Ar-H), 7.09 (s, 1H, -CH, 2-Pyridine), 7.67-7.70 (m, 4H, Ar-H), 7.86 (d, 2H, Ar-H), 8.42 (broad s, 2H, -NH_2), 9.07 (broad s, 1H, OH).

2-Amino-6-(1-(benzo[d]thiazol-2-yl)-3-methyl-5-oxo-4, 5-dihydro-1H-pyrazol-4-yl)-4-(4-methoxyphenyl) nicotinonitrile (**6e**): Color: Brown. Yield: 68% (A), 53% (B), 75% (C). M.p.: 132-134°C. FT-IR (KBr, ν, cm^{-1}): 3444, 3420 ν(NH_2), 3028 ν(ArH), 2933 ν(aliphatic, CH), 2222 ν(CN), 1701 ν(C=O, pyrazolone), 1635 ν(C=N), 1250 ν(OCH_3). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.09 (s, 3H, CH_3), 3.03 (s, 1H, CH methine), 3.84-3.89 (s, 3H, OCH_3), 6.98 (d, 2H, Ar-H), 7.12-7.18 (s, 1H, 2-Pyridine), 7.33-7.72 (m, 4H, Ar-H), 7.97 (d, 2H, Ar-H), 8.55 (broad s, 2H, NH_2).

2-Amino-6-(1-(benzo[d]thiazol-2-yl)-3-methyl-5-oxo-4, 5-dihydro-1H-pyrazol-4-yl)-4-(4-nitrophenyl) nicotinonitrile (**6f**): Color: Faint orange. Yield: 72% (A), 55% (B), 76% (C). M.p.: 227-229 °C (Dec.). FT-IR (KBr, ν, cm^{-1}): 3358, 3277 ν(NH_2), 3064 ν(ArH), 2929 ν(aliphatic, CH), 2218 ν(CN), 1719 ν(C=O, pyrazolone), 1636 ν(C=N), 1521, 1346 ν(NO_2). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.61 (s, 3H, CH_3), 2.98 (s, 1H, CH methine), 7.23 (s, 1H, 2-Pyridine), 7.56-7.86 (m, 4H, Ar-H), 8.13-8.18 (d, 2H, Ar-H), 8.20-8.26 (d, 2H, Ar-H), 8.35 (broad s, 2H, NH_2).

2.7. Pharmacology

2.7.1. Antimicrobial activity [32]

A preliminary antibacterial activity has been carried out according to Well Diffusion Method [33]: The antimicrobial activity of synthesized compounds have been studied *in-vitro* against four types of bacteria (*Staphylococcus aureus* and *Streptococcus Pneumoniae* as Gram positive bacteria, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* as Gram negative bacteria) obtained from laboratories of Department of Biology, College of Science, Al-Mustansiriyah University were clinical activated and maintained on nutrient agar medium. Type of media used: Mueller-Hinton agar for testing antibacterial activity. Ciprofloxacin and ceftriaxone were used as standard drugs for antibacterial activity. Tested compounds were dissolved in dimethylsulphoxide (DMSO) to give final four different concentrations of 500, 250, 125 and 62.5 µg/mL.

2.7.2. Sensitivity assay

Well diffusion assay was carried out by using bacterial suspension (1.5×10^8 CFU/mL) obtained from McFarland turbidity standard (number 0.5) [34]. This was used to inoculate by swabbing the surface of Mueller-Hinton agar (MHA) plates. The excess liquid was air-dried under a sterile hood. In each agar plate of tested bacteria five wells were made and (100 µL) of each concentration was added in it. The plates were transferred to be incubated at 37 °C for 24 hours.

3. Results and discussion

5-Pyrazolones (**3a**) was synthesized by cyclocondensation of compound **2a** with ethyl acetoacetate using conventional, microwave and ultrasound methods. 4-Acetyl-1-(benzo[*d*]thiazol-2-yl)-3-methyl-1*H*-pyrazol-5(4*H*)-one (**4a**) was synthesized by the acylation of compound **3a** with the corresponding acid chloride following Jensen's procedure and are good crystallizing compounds often obtainable both in the keto form by recrystallization from polar solvents as alcohol-water or dioxane-water mixtures, and in the enol form by recrystallization from non polar solvent as chloroform. [27].

The chalcone derivatives, (*E*)-1-(benzo[*d*]thiazol-2-yl)-4-(3-(4-(substituted) phenyl)acryloyl)-3-methyl-1H-pyrazol-5(4*H*)-one as chalcone derivatives (**5a-f**) were synthesized by reacting compound **4a** with different substituted aromatic aldehydes by using ammonium acetate in ethanol. Finally, the compounds **5a-f** were reacted with malononitrile to give corresponding compound **6a-j** in good yields.

The synthesized compounds were characterized by FT-IR and ^1H NMR techniques. The IR spectrum of chalcones (**5a-f**) exhibited characteristic band absorption for α,β-unsaturated ketone group in the region of 1677-1696 cm^{-1}. The absorptions bands at around 1588-1616 cm^{-1} were assigned to the existing of conjugated C=C. The aldole condensation of the final compounds (**6a-f**) exhibited very similar features and showed the expected bands for the characteristic groups which are present in the compounds, such as NH_2 and the CN stretching vibrations is an evidence of ring closure; it showed the appearance of multiple bands in at 3232 and 3444 cm^{-1} range due to stretching vibrations of NH_2 group, the spectrum exhibited characteristic new absorption band at 2171-2222 cm^{-1} range were assigned to stretching vibrations of CN group. In the proton NMR spectral data, all protons were seen according to the expected chemical shift and integral values. The aromatic protons appeared as multiplet peaks within the range δ 7.00-7.80 ppm. Singlet signals derived from -NH_2 and Ar-H of pyridine ring (**6a-f**) structure appeared at δ 8.40-9.07 and 6.97-7.09 ppm, respectively.

The reaction may proceed via imine formed from aldehyde and ammonium acetate, imine reacts with alkylidene malononitrile (from condensation of aromatic aldehyde with malononitrile) to give the following intermediate, carry on by cycloaddition, isomerization, aromatization to afford the 2-amino-3-cyanopyridine.

Bacterial growth and the antibacterial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the well in mm, and show that the zone of inhibition increased with the increasing of concentration of the tested compounds (Table 1).

Table 1. Antibacterial screening data (zone of inhibition in mm) for final compounds **6a-f**, 2-MBT, ciprofloxacin and ceftriaxone.

Compound	R	Conc. (µg/mL)	Inhibition zone (mm)			
			Staphylococcus aureus	*Streptococcus Pneumoniae*	*Klebsiella Pneumoniae*	*Pseudomonas aeruginosa*
6a	H	500.0	15	20	20	16
		250.0	14	18	17	16
		125.0	13	17	15	15
		62.5	11	17	13	13
6b	Cl	500.0	13	14	12	13
		250.0	11	11	13	11
		125.0	10	10	10	12
		62.5	10	10	10	-
6c	$N(CH_3)_2$	500.0	19	11	-	12
		250.0	18	11	12	11
		125.0	17	10	-	11
		62.5	13	9	10	10
6d	OH	500.0	11	20	18	-
		250.0	-	13	16	-
		125.0	-	12	14	-
		62.5	10	10	13	-
6e	OCH_3	500.0	-	20	11	13
		250.0	-	15	10	12
		125.0	10	16	-	10
		32.5	10	10	-	10
6f	NO_2	500.0	11	16	11	11
		250.0	-	14	-	10
		125.0	-	12	-	10
		62.5	9	10	9	-
2-MBT		500.0	-	13	15	13
		250.0	-	12	10	13
		125.0	-	11	10	12
		62.5	-	11	10	10
Ciprofloxacin		500.0	25	29	32	22
		250.0	25	28	34	19
		125.0	25	20	30	18
		62.5	25	15	29	13
		500.0	32	26	10	20
		250.0	28	19	-	19
Ceftriaxone		125.0	24	17	10	14
		62.5	24	16	4	9
DMSO		Pure	-	-	-	-

All tested compounds exert significant antibacterial activity in comparison to DMSO as control group and an interesting activity against gram positive *Staphylococcus aureus* unlike the parent compound.

In comparison to standard compound (ciprofloxacin), tested compound show lower activity against all tested bacterial species. While for the standard compound (ceftriaxone) the tested compounds showed better activity against *Klebsiella Pneumoniae* and lower antibacterial effect against the other three species. Finally compound **6a** derivative may regard the best one generally and the nitrobenzene (**6f**) was the least one.

4. Conclusion

The area of the synthesis of some novel 2-amino nicotinonitrile ring derivatives continues to grow, and better methods were developed for the synthesis of these interesting heterocycles to allow the discovery of new drug candidates. The advantages of microwave irradiation under solvent free condition method are high yields, relatively short reaction times, low cost and simple experimental.

Various new derivatives of 2-amino-6-(1-(benzo[*d*]thiazol-2-yl)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-4-yl)-4-((4-sub-sitituted)phenyl) nicotinonitrile (**6a-f**) were synthesized and screened for antibacterial activity, most of these compounds show good antimicrobial activity comparable with marketable compounds and among them hydroxy containing derivative is the only one which exhibited no antibacterial activity against *Pseudomonas aeruginosa* and benzaldehyde derivative is the best one against *Klebsiella pneumonia.*

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