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3-Oxobutanamides in Heterocyclic Synthesis: Synthesis, Antimicrobial and Antioxidant Activity of Pyridine, Thiophene, Diazepine and Thiazole Derivatives

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Abstract: In the last few years we and others reported a variety of synthesis of heteroaromatics that have been developed utilizing β–oxoanilides as readily obtainable compounds. In this study, treatment of 3-Oxobutanamides 1 with phenyl isocyanate at room temperature in basic medium and DMF afforded thiocarbamoyl derivative 3 through intermediate 2 upon treatment with dilute HCl. The non-isolable potassium salt 2 was allowed to react with α-halo carbonyl compounds such as ethyl chloroacetate and chloroacetonitrile in dry DMF at room temperature to furnished thiophene 6 and thiazole derivative 9 respectively. Compound 6 was treated with hydrazine hydrate in ethanol to yield thiophene-5-carbohydrazide derivative 7. Also, compound 1 reacted with malononitrile and sulfur element in refluxing absolute ethanol and triethylamine to yield thiophene derivative 11, which reacted with formic acid and α-chloro acetylchloride to give thieno[2,3-d]pyrimidine derivative 12 and chloro acetamide derivative 13. Treatment of compound 13 with ammonium thiocyanate led to thiazolo[3,2-a]thieno[2,3-d]pyrimidine derivative 15 through non-isolable 14. Also, condensation of 1 with malononitrile and ethylene diamine afforded the pyridine derivative 17 and 1,4-diazepine derivative 18. The structures and formulas of all the synthesized compounds were supported by spectral data. Some of the synthesized compounds were evaluated for their antibacterial and antioxidant activity. These compounds showed different degrees of activity.

Keywords: 3-oxo-butanamide, Thiophene, Thiazole, Thieno[2, 3-d]pyrimidine

1. Introduction

Fluorine containing compounds play a very important role for their applications in medicinal chemistry [1]. Fluorine being the most electronegative atom with small atomic radius helps to enhance the biological activity of newly designed molecules [2]. Fluorinated compounds have been widely used as important antifungal [3], anticancer [4] and antibacterial drugs [5]. Some of the fluorinated motifs have been utilized for treating cardiovascular and obesity related diseases [6]. A new series of potent GPR119 agonists, possessing fluorine atom as a substituent have been reported [7]. Henceforth, fluorine substitution remains a lucrative aspect in the development of bio-active drug molecules. In the light of these facts and in continuation of our interest in the synthesis of heterocycles [8-11] motif with fluorine atom substituted as $–CF_3$ at *m*-position in the phenyl ring.

2. Discussion

2.1. Chemistry

We report here the results of our study of the reaction of 3-oxo-N-(3-trifluoromethyl)-phenyl-butanamide 1 [12] with some electrophilic and nucleophilic reagents. Thus, the reaction of compound 1 with phenyl isothiocyanate in dry DMF at room temperature in basic medium led to the formation of the non-isolable intermediate 2 [13], which transferred to thiocarbamoyl derivative 3 upon treatment with dilute HCl. The structure 3 was confirmed on the basis of its elemental analysis and spectral data. The thiole form 3 was verified by its ^{1}H NMR spectrum which display a singlet signal at δ 12.68 ppm due to SH proton besides the other expected signals, The ^{13}C NMR of compound 3 showed a signals at: δ 30.66 ppm (q) for CH_3, δ 105.89 ppm (s), δ 120.70 ppm (d), δ 120.70 ppm (d), δ 124.82 ppm (d), δ 125.65 ppm (d), δ 125.65 ppm (d), δ 128.64 ppm (s), δ 130.13 ppm (d), δ 130.13 ppm (d), δ 130.31 ppm (d), δ 130.31 ppm (d), δ 137.88 ppm (s), δ 137.88 ppm (s), δ 152.04 ppm (s), δ 164.76 ppm (s), and the quaternary carbon at δ 175.52 ppm (s), δ 195.12 ppm (s) for the (2C=O) group, (Figure 1).

The non-isolable potassium salt 2 was allowed to react with α-halo carbonyl compounds such as ethyl chloroacetate at room temperature to give the thiophene derivative 6 and discard the thiazole structure 5 on the basis of analytical and spectral data. ^{1}H NMR spectrum of 6 showed the presence of a singlet signal at δ 2.56 ppm for methyl protons, triplet signal at δ 1.25 ppm for methyl ester, a quartet signal at δ 4.21 ppm characterized for methylene ester, singlet signals at δ=9.37, δ 10.38 ppm for two NH protons. ^{13}C NMR of 6 δ 14.73 ppm (q) for CH_3, δ 15.13 ppm (q), δ 60.52 ppm (t) for the methyl group, δ 110.00 ppm (s), δ 116.64 ppm (d), δ 116.64 ppm (d), δ 119.63 ppm (d), δ 119.63 ppm (d), δ 120.23 ppm (d), δ 120.62 ppm (d), δ 123.60 ppm (d), δ 123.94 ppm (d), δ 125.99 ppm (s), δ 125.99 ppm (s), δ 129.82 ppm (d), δ 130.13 ppm (d), δ 140.30 ppm (s), δ 142.08 ppm (s), δ 145.27 ppm (s), δ 155.17 ppm (s), δ 162.45 ppm (s, CO), δ 163.76 ppm (s, CO).

Formation of structure 6 was assumed to proceed via initial alkylation followed by interamolecular cyclization and loss of H_2O molecule to give thiophene derivative 6. Furthermore, elucidation of structure 6 come from its reaction with hydrazine hydrate to yield thiophene-5-carbohydrazide derivative 7. The ^{1}H NMR spectrum of compound 7 revealed a singlet signal at δ 2.45 ppm for CH_3 and three signals at δ 6.37, 9.10, 10.30 ppm for NH_2 and two NH protons, in addition to an aromatic and NH protons at δ 6.92-8.20 ppm, (Figure 1).

In contrast, when non-isolable 2 was stirred with chloro acetonitrile in dimethylformamide at room temperature may be formulated as thiazole 9 or thiophene 10. The structure 10 was ruled out and structure 9 was conformed based on its spectral data (IR and ^{1}H NMR). The IR spectrum of 9 revealed the presence of NH_2 group at ν_{max}=3455, 3288 and the absence of CN group absorption band, ^{1}H NMR spectrum revealed a singlet signal at δ 2.43 ppm assigned to CH_3, a multiplet signals at 7.07-8.18 corresponding to aromatic protons, NH_2, thiazole-H, and singlet signal at δ 9.63 ppm assigned to NH group, (Figure 1).

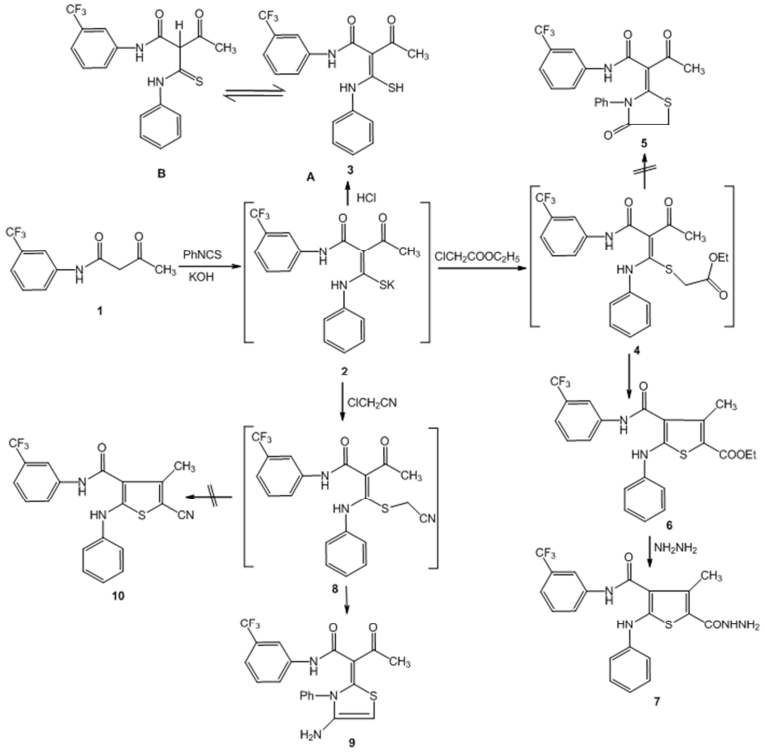

Figure 1. *Synthesis of thiophene and thiazole derivatives.*

The reactivity of 3-oxobutanamide 1 towards active methylene reagents was investigated, thus, it has been found that 3-oxo-butanamide 1 reacted with mixture of malononitrile and sulfur element according to Gewald methodology, yielded the accepted thiophene derivative 11 which established based on its elemental analysis and spectral data (IR, ^{1}H NMR). IR spectrum showed the presence of absorption band at 3391, 3321 cm^{-1} for NH_2 group and 2222 cm^{-1} for CN group. The ^{1}H NMR spectrum showed a singlet signal at δ 2.37 ppm assigned to CH_3, singlet signal at δ 6.86 ppm assigned to NH_2, singlet signal at δ 9.38 ppm assigned to NH, in addition to aromatic multiplet protons at δ 7.74-7.65 ppm. Treatment of compound 11 with formic acid on reflux gave the thieno[2,3-*d*]pyrimidine derivative 12. The IR spectrum of the reaction product 12 revealed the disappearance cyano group absorption band, showed absorption at ν 3264, 3150, 1694, 1668 cm^{-1} corresponding to 2NH and 2C=O function respectively. Its ^{1}H NMR spectrum showed singlet signal at δ 2.82 for CH_3, signals at δ 10.46 and 12.54 ppm due to two NH protons, in addition to an aromatic multiplet protons in the region δ 7.46-8.18 ppm (Figure 2).

Furthermore, reaction of thiophene derivative 11 with chloro acetylchloride in DMF at room temperature gave chloro acetamide derivative 13. Hetero cyclization of 13 was performed on refluxing ethanol in the presence of ammonium thiocyanate led to the non-isolable thiophenylimino-1,3-thiazolodin-4-one 14 followed by cyclization to thiazolo[3,2-*a*]thieno[2,3-*d*]pyrimidine derivative 15 [14, 15]. Compound 15 was confirmed based on its elemental analysis and spectroscopic data. Thus, its IR spectrum showed disappearance of CN group and appearance of bands of two NH group at ν 3358, 3128 cm^{-1} and ν 1711, 1666 cm^{-1} for the two carbonyl functions. Moreover, ^{1}H NMR revealed a singlet signal at δ 2.74 ppm assigned to CH_3, a singlet signal at δ 4.01 ppm assigned to CH_2, in addition to an aromatic multiplet in the region δ 7.10-7.96 ppm, and two singlet signals at δ 9.21, 10.56 ppm for 2NH groups, (Figure 2).

Also, the pyridine derivative 17 was obtained by reaction of 3-oxobutanamide 1 with malononitrile in ethanolic piperidine. The structure 17 was readily confirmed based on spectroscopic data. The IR spectrum of the reaction product 17 revealed the presence of cyano group at ν 2205 cm^{-1}, NH_2 group at ν 3485, 3319 cm^{-1}, carbonyl group at ν 1682 cm^{-1}. ^{1}H NMR spectrum revealed a singlet signal at δ 2.20 ppm assigned to CH_3, a singlet signal at δ 5.69 ppm assigned to pyridine-H, and singlet signal at δ 6.93 ppm assigned to NH_2 group, in addition of the aromatic protons in the molecule, (Figure 2).

Finally, when 3-oxobutanamide 1 condensed with ethylenediamine in ethanol and presence few drops of acetic acid to afford 1,4-diazepine derivative 18, the chemical structure of compound 18 was established on the basis of its elemental analysis and spectral data. The IR spectrum of compound 18 showed disappearance of two carbonyl groups. ^{1}H NMR spectrum showed a singlet signal at δ 1.95 ppm assigned to CH_3, a triplet signals at δ 3.15 ppm corresponding to CH_2 and multiplet signals at δ 3.39 ppm corresponding to CH_2, a singlet signal at δ 4.58 ppm assigned to diazepine-H, a singlet signal at δ 9.21 ppm corresponding to NH group, in addition to aromatic multiplet protons in the region δ 7.21-8.14 ppm, (Figure 2).

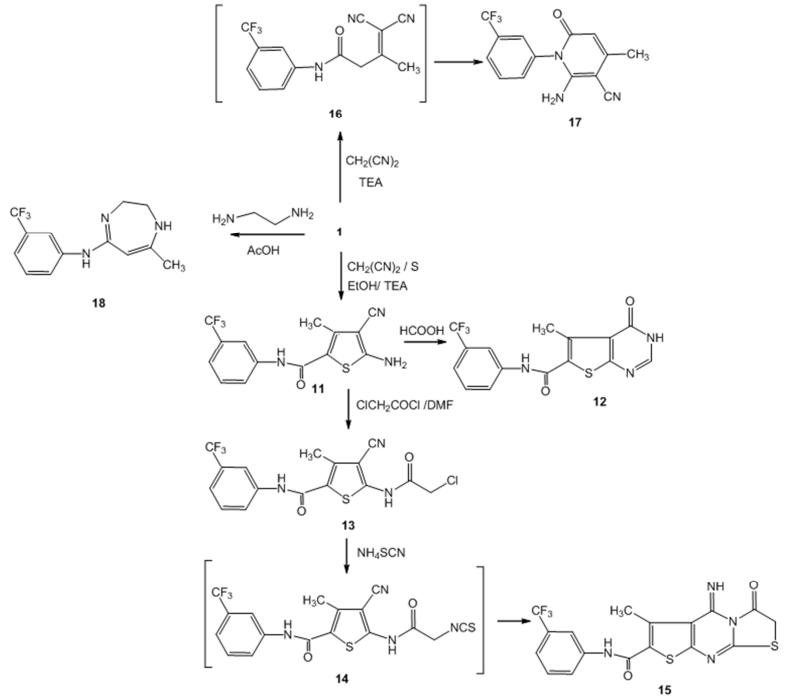

Figure 2. *Synthesis of pyridine, diazepine and condensed thiophene derivatives.*

2.2. Antimicrobial Activities

2.2.1. Test Microorganisms

Test organisms used in this study included strains were Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*). The tested fungal strains and isolates were *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Alternaria alternata*, and *Fusarium oxysporum* obtained from Mycology Lab., Botany and Microbiology Department, Faculty of Science, Al-Azhar University (Assiut Branch), Egypt.

2.2.2. Agar Well Diffusion Method

The antimicrobial activities were carried out by well diffusion method [16]. Well diameter was 8 mm filled with 50 µl of the 1% (w/v) test samples. Chloramphenicol (1 mg/ml) was used as positive control for bacteria and nystatine (0.1%) for fungi, while dimethylsulfoxide (DMSO) was used as negative control. Muller-Hinton agar (MHA) plates previously inoculated with 24 h old broth cultures of the bacterial strains were used for antibacterial activity. 1% glucose-Czapek's (Cz) plates previously inoculated with a spore suspension of molds were used for antifungal activity. The diameter of the inhibition zone around the well, measured in millimeter, is used as positive bioactivity.

2.2.3. DPPH Radical Scavenging Activity

The antioxidant activity of test samples was measured in the form of hydrogen donating or scavenging activity according to the procedure of [17] with some modifications. The color of the reaction mixture changes from purple to yellow with decreasing of absorbance at wavelength 517 nm by DPPH radical scavenged. 1.8 ml of 0.1 mM DPPH (Sigma-Aldrich, Germany) (4 mg/100 ml of methanol) solution was added to 0.2 ml of the test sample in methanol at different concentrations (1, 0.5 and 0.1 mg/ml) and the blank. This setup was left at room temperature for 30 minutes (shaken vigorously between) and absorbance was measured at 517 nm by using spectrophotometer (JENWAY 7315). Butylated hydroxytoluene (BHT) was used as the positive control and experiment was done in triplicate. The capacity to scavenge the DPPH radical was calculated using the following formula:

$$\%\ \text{DPPH radical scaveng}_{\text{ing}} = \frac{A - B}{A} \times 100$$

Where, A is the absorbance of the negative control (DPPH and methanol) and B is the absorbance of the sample (DPPH, methanol and sample).

2.2.4. Statistical Analysis

Data were recorded as the mean ± standard deviation of the results in triplicate. All statistical analysis was performed using one-way analysis of variance (ANOVA). Differences between means were considering using Duncan's New Multiple Range Test (DMRT) at the significance level of 0.05.

2.2.5. Results

Antimicrobial activities

The study deals with the antibacterial and antifungal effects of compounds (TFs). The antibacterial activities of these extracts were estimated on the growth of five pathogenic bacteria representing two Gram-positive bacteria (*B. subtilis*, and *S. aureus*) and three Gram-negative bacteria (*E. coli*, *K. pneumonia* and *P. aeruginosa*). While, the antifungal activities of these extracts were evaluated against five fungal species (*A. flavus*, *A. fumigatus*, *A. niger*, *A. alternata*, and *F. oxysporum*) using well diffusion method.

According to the results, the antibacterial activities of tested compounds showed different degrees of inhibition against tested bacteria. Compounds (1 and 3) exhibited antibacterial activities against *B. subtilis* and *K. pneumonia* in comparison to chloramphenicol, while compounds (11 and 13) showed low significant antibacterial activity on *P. aeruginosa*. Compounds (7, 11 and 9) showed less significant inhibition against tested bacterium *B. subtilis* as well as compound (12) against *K. pneumonia*. *Bacillus subtilis* was most sensitive to tested compounds followed by *K. pneumonia* and *P. aeruginosa*. *Escherichia coli* and *S. aureus* were resistant to all tested compounds. On the other hand, all tested bacteria were not affected by the applied compounds 17, 6, 15 and 18 (Table 1).

The antifungal impact of tested (TFs) showed that fungi were more resistant/not sensitive to all compounds tested in comparison to nystatin (Table 2).

Table 1. *Antibacterial activity – well diffusion method – well diameter 8 mm filled with 50 µl of conc. 10 mg/ml.*

Compound	Inhibition zone diameter (mm) including well				
	Gram +ve		Gram –ve		
	B. subtilis	*S. aureus*	*E. coli*	*K. pneumonia*	*P. aeruginosa*
12	19.33±0.88a	-	-	19.66±0.88a	-
3	18.00±057a	-	-	20.330.88a	-
17	-	-	-	-	-
7	12.33±0.88c	-	-	-	-
11	14.66±0.33b	-	-	-	12.66±0.88a
6	-	-	-	-	-
15	-	-	-	-	-
18	-	-	-	-	-
13	-	-	-	-	11.66±0.88a
9	16.33±0.66b	-	-	15.66±0.33b	-

Compound	Inhibition zone diameter (mm) including well				
	Gram +ve		Gram –ve		
	B. subtilis	*S. aureus*	*E. coli*	*K. pneumonia*	*P. aeruginosa*
Chloramphenicol +ve control (0.1%)	20.33±0.33a	16.33±0.66	14.00±0.57	18.33±0.66a	13.66±0.88a
DMSO -ve control	-	-	-	-	-

The data are given as averages of three replicates ± standard error. Values followed by the different letters are significantly different at $p < 0.05$.

Table 2. *Antifungal activity. – well diffusion method – well diameter 8 mm filled with 50 µl of conc. 10 mg/ml.*

Compound	Inhibition zone diameter (mm) including well				
	Aspergillus flavus	*Aspergillus fumigatus*	*Aspergillus niger*	*Alternaria alternata*	*Fusarium oxysporum*
All tested compounds	-	-	-	-	-
+ve control Nystatine (0.1%)	14.00±0.57	16.33±0.66	14.66±0.33	16.00±057	13.66±0.33
DMSO -ve control	-	-	-	-	-

2.3. Antioxidant Activity

The *in vitro* antioxidant activities of 10 compounds were determined through the comparison of the standard BHT using DPPH assay and the results are presented in Table 3. Compound (6) at concs. 1, 0.5 and 0.1 mg/ml revealed a remarkable free radical scavenging effect with an inhibition value of 85.90±0.43%, 84.06±0.09% and 55.15±0.53% respectively, and its efficacy was homogenous with BHT. Other tested compounds showed different degrees of activity as compared to BHT at $p<0.05$.

Table 3. *Antioxidant activity (% DPPH radical scavenging).*

	Compound	*Conc. 1 mg/ml*	*Conc. 0.5 mg/ml*	*Conc. 0.1 mg/ml*
1	7	42.61±0.35g	25.15±0.70g	9.55±3.15d
2	17	19.22±0.46L	4.85±0.32L	4.07±21e,f
3	3	80.08±0.20b	73.23±0.45c	38.34±0.43b
4	6	85.90±0.43a	84.06±0.09a	55.15±0.53a
5	9	36.56±0.46h	29.10±0.30e	3.96±0.65e,f
6	11	75.38±35c	55.12±0.54d	32.40±0.23c
7	18	48.15±0.61e	14.74±0.09j	5.24±1.80e
8	12	6.70±0.56k	4.07±0.30L	2.61±1.05e,f
9	13	45.49±0.53f	23.45±0.55h	5.92±0.50e
10	15	26.79±0.69i	16.34±0.33i	3.08±0.30e,f

The data are given as averages of three replicates ± standard error. Values followed by the different letters are significantly different at $p < 0.05$.

3. Experimental

All melting points are uncorrected. IR spectra (KBr) were recorded on a FTIR 5300 spectrometer (ν, cm^{-1}). The ^1H and ^{13}C NMR spectra were recorded at 500 MHz on a Broker NMR spectrometer (δ, ppm) using TMS as an internal standard. Mass spectra were obtained on JEOL JMS600 H Root mass spectrometer at 70 ev. Elemental analysis was carried out by the Micro analytical Research Center, Faculty of Science, Cairo University and Micro analytical Research Center, Assiut University and Brucker Company in Switzerland Center.

2-(Mercapto-phenylamino-methylene)-3-oxo-N-(3-trifluoromethyl-phenyl)-butanamide (3)

To a stirred solution of potassium hydroxide (0.01 mol) in dimethylformamide 20 ml, compound 1 (0.01 mol) was added to the mixture and stirring was continued for 6 h. Then poured over crushed ice containing hydrochloric acid. The solid product formed was filtered off, washed with water, dried, and recrystallized from ethanol to afford 3 as dark yellow crystals, yield 50%, mp=135 °C. IR (KBr) ν: 3241, 3199 (2NH); 1694, 1603 (2C=O) cm^{-1}. ^1H NMR (DMSO-d6): 2.66 (s, 3H, CH_3), 6.96-7.54 (m, 11H, Ar-H + NH + SH) and 12.68 (s, 1H, NH) δ ppm. ^{13}C NMR δ C: 30.66 (q); 105.89 (s); 120.70 (d); 120.70 (d); 124.82 (d); 125.65 (d); 125.65 (d); 128.64 (s); 130.13 (d); 130.13 (d); 130.31 (d); 130.31 (d); 137.88 (s); 137.88 (s); 152.04 (s); 164.76 (s); 175.52 (s, CO); 195.12 (s, CO). Anal. Calcd. for $C_{18}H_{15}F_3N_2O_2S$ (380.39): C, 56.84; H, 3.97; F, 14.98; N, 7.36; O, 8.41; S, 8.43%. Found: C, 56.80; H, 3.90; F, 14.95; N, 7.33; O, 8.40; S, 8.40%.

3-Methyl-5-phenylamino-4-(3-trifluoromethyl-phenylcarbamoyl)-thiophene-2-carboxylic acid ethyl ester (6)

To a stirred solution of potassium hydroxide (0.01 mol) in dimethylformamide 20 ml, compound *1* (0.01 mol) was added to the mixture and stirring was continued for 6 h. Then ethyl chloroacetate (0.01 mol) was added drop wise over a period of 30 min., after the addition was complete, the reaction mixture was stirred for additional 24 h. Then it was poured over crushed ice. The solid product formed was filtered off, washed with water, dried, and finally recrystallized from ethanol afford 6 as yellow crystals, yield 65%, mp=122 °C. IR (KBr) ν 3456 (2NH), 1701, 1624 (2CO)

cm^{-1}. ^1H NMR (DMSO-d_6) δ ppm: 1.25 (t, 3H, CH_3) 2.56 (s, 3H, CH_3), 4.21 (q, 2H, CH_2) 7.06-8.22 (m, 9H, Ar-H), 9.37 (s, 1H, NH) and 10.38 (s, 1H, NH) δ ppm. ^{13}C NMR δ C=14.73 (q); 15.13 (q); 60.52 (t); 110.00 (s); 116.64 (d); 116.64 (d); 119.63 (d); 119.63 (d); 120.23 (d); 120.62 (d); 123.60 (d); 123.94 (d); 125.99 (s); 125.99 (s); 129.82 (d); 130.13 (d); 140.30 (s); 142.08 (s); 145.27 (s); 155.17 (s); 162.45 (s, CO); 163.76 (s, CO). Anal. Calcd. For $C_{22}H_{19}F_3N_2O_3S$ (448.47): C, 58.92; H, 4.27; F, 12.71; N, 6.25; O, 10.70; S, 7.15%. Found: C, 58.91; H, 4.25; F, 12.70; N, 6.23; O, 10.68; S, 7.13%.

5-Hydrazinocarbonyl-4-methyl-2-phenylamino-thiophene-3-carboxylic acid (3-trifluoromethyl-phenyl)-amide (7)

A mixture of compound 6 (0.01 mol) and hydrazine hydrate (5 mL) in ethanol (20 ml) was refluxed for 10 h. The reaction mixture was poured onto ice-cold water, filtered off, recrystallized from ethanol to give 7 as gray crystals in yield 45%, mp=180°C. IR (KBr) ν 3430, 3283, 3198 (3NH, NH_2), 1701, 1644 (2C=O) cm^{-1}. ^1H NMR (DMSO-d6): 2.45 (s, 3H, CH_3), 6.92-8.20 (m, 12H, Ar-H + NH), 8.37 (s, 2H, NH_2), 9.10 (s, 1H, NH), and 10.30 (s, 1H, NH) δ ppm. Anal. Calcd. For $C_{20}H_{17}F_3N_4O_2S$ (434.44): C, 55.29; H, 3.94; F, 13.12; N, 12.90; O, 7.37; S, 7.38%. Found: C, 55.27; H, 3.92; F, 13.10; N, 12.89; O, 7.35; S, 7.36%.

2-(4-Amino-3-phenyl-3H-thiazolo-2-ylidene)-3-oxo-N-(3-trifluoromethyl-phenyl)-butanamide (9)

To a solution of potassium hydroxide (0.01 mol) in dimethylformamide 20 ml, compound 3 (0.01 mol) was added. The mixture was stirred for 6 h, then chloro acetonitrile (0.01 mol) was added drop wise over a period of 30 min. after the addition was complete, the reaction mixture was stirred for additional 24 h., then it was poured over crushed ice. The solid product formed was filtered off, washed with water, dried, and finally recrystallized from ethanol to afford 9 as brown crystals, yield 70%, mp=170°C. IR (KBr) ν 3455, 3288 (NH, NH_2), 1702, 1647 (2C=O) cm^{-1}. ^1H NMR (DMSO-d_6): 2.43 (s, 3H, CH_3), 7.07-8.18 (m, 12H, Ar-H + NH_2 + thiazole-H) and 9.63 (s, 1H, NH) δ ppm. Anal. Calcd. For $C_{20}H_{16}F_3N_3O_2S$ (419.43): C, 57.27; H, 3.85; F, 13.59; N, 10.02; O, 7.63; S, 7.64%. Found: C, 57.20; H, 3.80; F, 13.60; N, 10.00; O, 7.60; S, 7.60%.

5-Amino-4-cyano-3-methyl-thiophene-2-carboxylic acid (3-trifluoromethyl-phenyl)-amide (11)

To a solution of oxobutanamide 1 (0.01 mol), sulfur element and malononitrile and few drops of triethylamine in absolute ethanol 30 ml was refluxed for 5 h., the reaction mixture left to cool and poured into ice-cold water and acidified with dilute HCl 10%, the solid product crystallized from ethanol to give 11 as brown crystals in yield 80%, mp=200 ºC. IR (KBr) ν: 3391, 3323 (NH_2), 3207 (NH), 2222 (CN), 1640 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6); 2.37 (s, 3H, CH_3), 6.86 (s, 2H, NH_2), 7.47-7.65 (m, 4H, Ar-H) and 9.38 (s, 1H, NH) δ ppm. Anal. Calcd. For $C_{14}H_{10}F_3N_3OS$ (325.31): C, 51.69; H, 3.10; F, 17.52; N, 12.92; O, 4.92; S, 9.88%. Found: C, 51.67; H, 3.08; F, 17.50; N, 12.90; O, 4.90; S, 9.84%

5-Methyl-4-oxo-3,4-dihydro-thieno[2,3-d]pyrimidine-6-carboxylic acid (3-triflu-oromethyl-phenyl)-amide (12)

A mixture of 11 (0.01 mol), and formic acid 10 ml was refluxed for 12 h., then left to cool the solid product formed was filtered, washed with water, dried and recrystallized from ethanol to give 12 as brown crystals, yield 45%, mp=275°C. IR (KBr) ν: 3264 (2NH), 1694, 1668 (2C=O) cm^{-1}. ^1H NMR (DMSO-d_6): 2.82 (s, 3H, CH_3), 7.46-8.18 (m, 5H, Ar-H+ pyrimidine-H), 10.46 (s, 1H, NH) and 12.54 (s, 1H, NH) δ ppm. ^{13}C NMR δ C=15.52 (q); 117.04 (d); 120.80 (d); 123.97 (d); 125.90 (s); 127.53 (s); 127.53 (s); 129.84 (d); 130.37 (d); 138.00 (s); 139.82 (s); 148.00 (s); 158.73 (s); 161.57 (s, CO); 164.93 (s, CO). Anal. Calcd. for $C_{15}H_{10}F_3N_3O_2S$ (353.33): C, 50.99; H, 2.85; F, 16.13 N, 11.89; O, 9.06; S, 9.07%. Found: C, 50.97; H, 2.83; F, 16.11 N, 11.87; O, 9.04; S, 9.05%.

5-(2-Chloro-acetylamino)-4-cyano-3-methyl-thiophene-2-carboxylic acid (3-trifluoromethyl-phenyl)-amide (13)

To a stirred solution of 11 (0.01 mol) in dimethylformamide 20 ml chloro acetylchloride (0.01 mol) was added drop wise over a period of 30 min. the resulting mixture was continued stirring for 3 h, then it was poured over crushed ice, the solid product formed was filtered off, washed with water dried and finally recrystallized from ethanol to give 13 as brown crystals, yield 50%; mp=175 °C. IR (KBr) ν: 3313 (2NH), 2224 (CN), 1705, 1654 (2C=O) cm^{-1}. ^1H NMR (DMSO-d_6): 2.51 (s, 3H, CH_3), 4.55 (s, 2H, CH_2), 7.44-8.12 (m, 4H, Ar-H), 10.32 (s, 1H, NH) and 12.34 (s, 1H, NH) δ ppm. Anal. Calcd. for $C_{16}H_{11}ClF_3N_3O_2S$ (401.80): C, 47.83; H, 2.76; Cl, 8.82; F, 14.19; N, 10.46; O, 7.96; S, 7.98%. Found: C, 47.81; H, 2.74; Cl, 8.80; F, 14.17; N, 10.44; O, 7.94; S, 7.96%.

5-Imino-6-methyl-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]thieno[2,3-d]pyrimidine-7-carboxylicacid(3-trifluoromethyl-phenyl)-amide (15).

To a solution of 13 (0.01 mol) in absolute ethanol 30 ml, ammonium thiocyanate and few drops of triethylamine was refluxed for 3 h. the solvent is removed by evaporation, the solid product so formed was collected by filtration and recrystallized from ethanol to give 15 as grey crystals, yield 40%, mp=190°C. IR (KBr) ν: 3499, 3358 (2NH), 1711, 1666 (2C=O) cm^{-1}. ^1H NMR (DMSO-d_6); 2.74 (s, 3H, CH_3), 4.01 (s, 2H, CH_2), 7.10-8.16 (m, 5H, Ar-H+ NH) and 10.56 (s, 1H, NH) δ ppm. Anal. Calcd. For $C_{17}H_{11}F_3N_4O_2S_2$ (424.43): C, 48.11; H, 2.61; F, 13.43; N, 13.20; O, 7.54; S, 15.11%. Found: C, 48.10; H, 2.60; F, 13.41; N, 13.18; O, 7.52; S, 15.10%.

2-Amino-4-methyl-6-oxo-1-(3-trifluoromethyl-phenyl)-1,6-dihydro -pyridine-3-carbonitrile (17).

A mixture of 1 (0.01 mol), malononitrile (0.01 mol) in ethanol 30 ml was treated with a few drops of triethylamine and refluxed for 4 h., the solid product which produced on hot was collected by filtration and recrystallized from ethanol to give 17 as colorless crystals, mp=235 °C. IR (KBr) ν: 3485, 3319 (NH_2); 2205 (CN); 1682 (C=O) cm^{-1}; ^1H NMR (DMSO-d6): 2.20 (s, 3H, CH_3), 5.69 (s, 1H, pyridine-H), 6.93 (s, 2H, NH_2) and 7.56-7.84 (m, 4H, Ar-H) δ ppm. Anal. Calcd. For $C_{14}H_{10}F_3N_3O$ (293.25): C, 57.34; H, 3.44; F, 19.44; N, 14.33; O, 5.46%. Found: C, 57.32; H, 3.42; F, 19.42; N, 14.32; O, 5.45%.

(7-Methyl-3,6-dihydro-2H-[1,4]diazepin-5-yl)-(3-trifluoromethyl-phenyl)-amine (18).

A mixture of 1 (0.01 mol), ethylenediamine (0.01 mol) in ethanol 30 ml was treated with a few drops of acetic acid and refluxed for 4 h., the solid product which produced on hot was collected by filtration and recrystallized from ethanol to give 18 as white crystals, mp=202°C. IR (KBr) ν: 3325 (NH); 1625, 1607 (2C=N) cm^{-1}; ^1H NMR (DMSO-d_6); 1.95 (s, 3H, CH_3), 3.15 (t, 2H, CH_2), 3.39 (m, 2H, CH_2), 4.58 (s, 2H, diazepin-H), and 7.21-8.14 (m, 4H, Ar-H) and 9.21 (s, 1H, NH) δ ppm. Anal. Calcd. For $C_{13}H_{14}F_3N_3$ (269.27): C, 57.99; H, 5.24; F, 21.17; N, 15.61%. Found: C, 57.97; H, 5.20; F, 21.13; N, 15.60%.

4. Conclusions

In summary, we have illustrated an oxobutanamides approach for the synthesis of a new library of polyfunctionalized heterocyclic compounds. In this protocol, oxobutanamides was reacted with some electrophilic and nucleophilic reagents, such as α-halo carbonyl compounds, malononitrile, ethylene diamine. The antibacterial activity against gram-positive bacteria, gram-negative bacteria, antifungal activity and antioxidant activity were studied.

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