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Design and synthesis of 3-pyrazolyl-thiophene, thieno[2,3-*d*]pyrimidines as new bioactive and pharmacological activities

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ABSTRACT

Two series of 5-ethyl-2-amino-3-pyrazolyl-4-methylthiophenecarboxylate and 2-thioxo-N^3-aminothieno[2,3-*d*]pyrimidines were prepared from 3,5-diethyl-2-amino-4-methylthio-phenecaboxylate and evaluated as anti-inflammatory, analgesic and ulcerogenic activities. Among the compounds studied, compounds which containing the substituted hydrazide at C-3 position **7**, **16**, and **17a** showed more potent anti-inflammatory and analgesic activities than the standard drug (Indomethacin and Aspirin), along without ulcerogenity. While compounds **2**, **5**, **9**, **10**, and **11c** showed moderate activities. Some of the newly synthesized compounds have good to excellent antimicrobial activity.

Thiophene-3-ethylcarboxylate derivative represent important building blocks in organic and medicinal chemistry. In addition, they are interest in their own right, due to their pharmacological properties. For example, 5-substituted 2-aminothiophenes and 2-amino-5-bromo-4-(3-nitrophenyl)thiophene are A_1 adenosine receptor agonists.[1,2] Also, many thieno[2,3-*d*]pyrimidine derivatives were covered by patents as phospho-diesterase inhibitors[3] and various receptor antagonists.[4,5] Various thieno[2,3-*d*]pyrimidine derivatives show pronounced anti-tumor[6] and radioprotective activities.[7] Based on thieno[2,3-*d*]pyrimidine derivatives, immunomodulators[8] and compounds used for prophylaxis and therapy of cerebral ischemia,[9] malaria,[10–12] tuberculosis,[13] Alzheimer's disease,[14] Parkinson's disease,[15] and other diseases were designed.[16,17]

As a part of our continuing program on the synthesis of anti-inflammatory and analgesic compounds as therapeutics agents, we have earlier reported a series of heterocyclic moieties that have biological and pharmacological activities.[18,19] In the library screening of our new compounds as anti-inflammatory and analgesic assay system, several thiophenes and thieno[2,3-*d*]pyrimidines provided inhibitory activity of inflammation even through some others five or focused heterocycle rings showed low anti-inflammatory and analgesic activity.

This report deals with the synthesis and the pharmacological evaluation of a series of 5-ethyl-2-amino-4-methylthiophenecarboxylate substituted at the C-3 position and thieno-[2,3-*d*]pyrimidines substituted at C-2 with various groups. The interaction of ethylacetoacetate with ethyl cyanoacetate and sulfur metal in ethanol medium in the presence of diethylamine led to ethyl thiophene-3-carboxylate **1**.[20] The hydrazide **2** obtained by refluxing of ethyl carboxylate **1** with hydrazine hydrate in ethanol medium, was used as precursor for the synthesis of 3-(pyrazolyl)-4-methylthiophenes and 3-(1,3,4-oxa/thiadiazol-2-yl)-4-methyltiophene as well as for the preparation of thieno[2,3-*d*]pyrimidines.

Hydrazide **2** undergo different cyclization reactions to give five member heterocycle compounds. The product of cyclization depends on the reagent used. This cyclization leads to the formation of 3-[(pyrazolyl derivatives)-carbonyl]-4-methylthiophene derivatives **3–5**, 3-(1,3,4-oxa/thiadiazol-2-yl)-4-methyltiophene derivatives **8a, b** and thieno[2,3-*d*]pyrimidines derivatives **10**, **14**, and **15**. The reaction of hydrazide **2** with α,β-diketones resulted in 2-amino-5-carboxyethyl-3-[(3,5-dimethylpyrazolyl derivatives)-carbonyl]-4-methylthiophene **3a, b**. The structure of the products was assigned on the basis of their spectral analyses.[21] The ^{1}H NMR spectrum of **3a**, as an example showed the signals at δ 1.30 (t, 3H, J = 7.0 Hz, CH_3), 2.19 (s 3H CH_3), 2.51(s, 3H, CH_3), 2.67 (s, 3H, CH_3), 4.25 (q, 2H, J = 7.10 Hz, CH_2), 6.21 (s, 1H, pyrazole proton), and 7.98 (br, 2H, NH_2, D_2O exchangeable), while the pyrazole proton disappeared in the ^{1}H NMR of **3b**. Also, the hydrazide 2 reacted with diethylmalonate afforded 5-ethyl-2-amino-3-[(3,5-dioxopyrazo-lidine-1-yl)-carbonyl]-4-methylthiophenecarboxylate (**5**).

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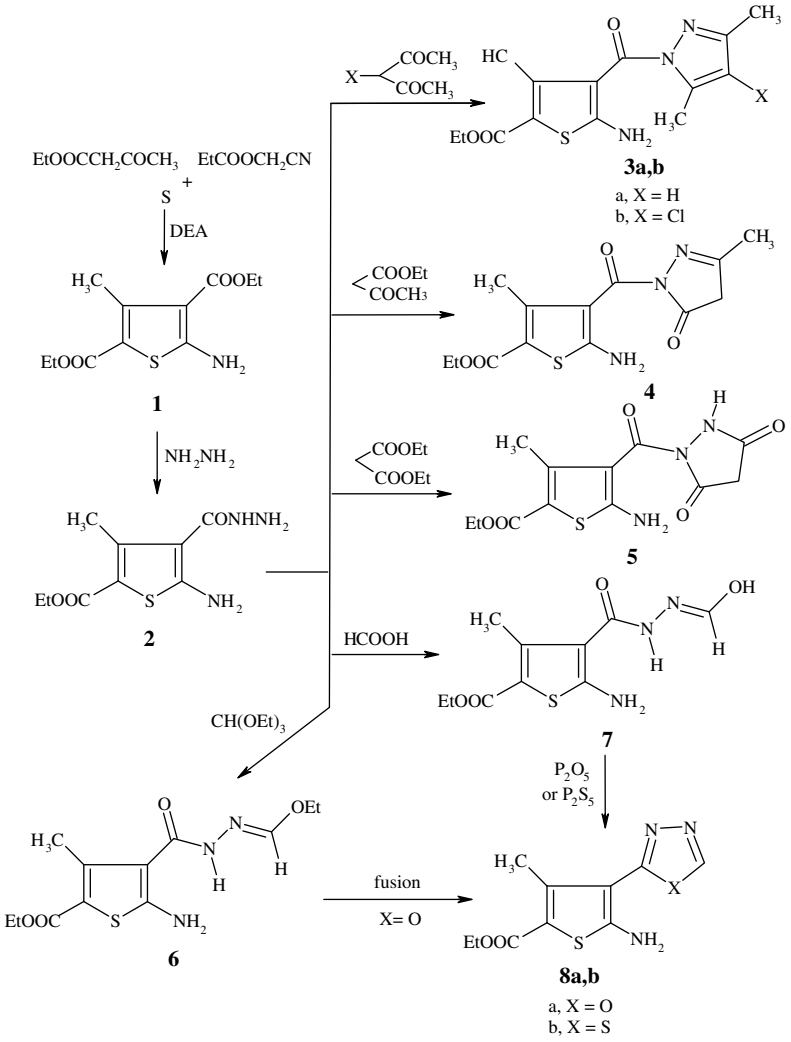
Treatment of compound **2** with formic acid yielded (*N*-hydroxymethylene-carbohydrazide) derivative (**7**) which undergoes cyclization to give 2-amino-5-carboxyethyl-3-(1,3,4-oxa/thiadiazol-2-yl)-4-methyltyhiophene (**8a, b**) when treated with P_2O_5 or P_2S_5. Not only that but give the same compound (**8a**) when compound **2** was treated with triethylorthoformate to afford 5-ethyl-2-amino-3-(*N*-ethoxymethylene-carbohydrazide)-4-methyl-thiophene-carboxylate (**6**) which undergoes cyclization by fusion to give the same compound (Scheme 1).

Scheme 2 reports cyclization procedure for the formation of 3-amino-2,5-dimethyl-6-carboxyethyl-thieno[2,3-*d*]pyrimidin-4-one (**10**) from compound **9**. Compound **1** was treated with acetic anhydride to afford 3,5-diethyl-2-acetylamino-4-methylthiophene-dicarboxylate (**9**). This cyclization realized on treatment with hydrazine hydrate.

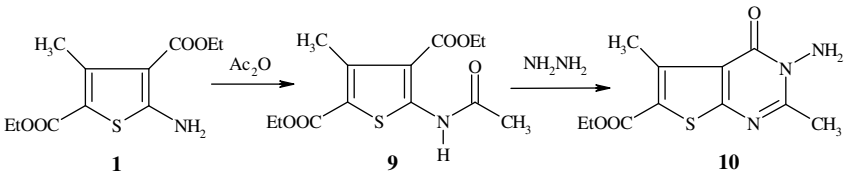
Also, treatment of compound **2** with aromatic aldehydes yielded arylmethylene hydrazide derivatives (**11a–c**). The cyclization of **11a–c** with acetic anhydride to afford **12a–c** was unsuccessful, while compound **11a–c** reacted with acetic anhydride to afford 5-ethyl-2-acetylamino-3-(arylmethylene hydrazide)-4-methyl-thiophenecarboxylate (**13a–c**) which undergoes cyclization upon refluxing in sodiumethoxide solution to afford 6-carboxyethyl-2,5-dimethylthieno[2,3-*d*]pyrimidin-(3*H*)-4-one (**14**) (Scheme 3), which characterized by ^{1}H NMR which revealed the disappearance of azomethine proton and the protons of aromatic substitutions. Also, compound **2** was treated with carbon disulfide to afford the promising compound 3-amino-6-carboxyethyl-5-methyl-2-thioxothieno[2,3-*d*]pyrimidin-4-one (**15**).

Finally, compounds **16** was formed by the reaction of compound **2** with potassium thiocyanate, the reaction was carried out by heating the mixture in 10% HCl, then the product undergoes cyclization when treated with chloroaceton or phenacyl bromide to afford 5-ethyl-2-amino-3-(4-methyl/or 4-phenyl-1,3-thiazol-2-yl)carbohydrazide-4-methyl-thiophene carboxylate (**17a**, **b**) (Scheme 4).

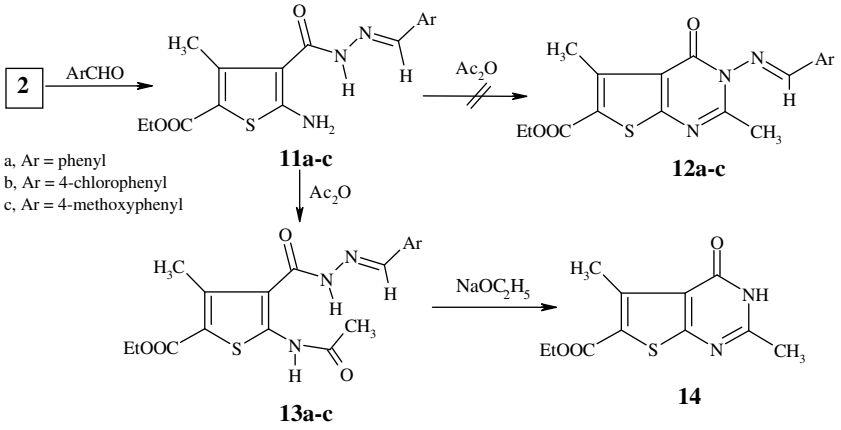
Amongst the compounds **2**–**17a** screened for antimicrobial activity, compound **7** and **17a** showed the highest activity against all bacteria. It exhibited stronger activity than ampicillin also towards *Bacillus cereus* and *Salmonella typhi* it was followed by compound **16** which showed the highest activity against *B. cereus* and *S. typhi*. As far as antifungal activity is concerned, all compounds showed good to excellent activity against all the fungi. Compounds **7** and **10** exhibited stronger activity than nystatin against *Alternaria alternata* and *Coillectotrium corchori*, compounds **7** and **17a** against *Macrophomina phaseolina*, compounds **7**, **15**, and **17a** against *Fusarium equiseti*. Compounds **2**–**15** were either inactive or moderately to fairly active against the tested bacteria whereas compounds **2**, **5**, and **9** exhibited good to excellent results against all the fungi. Introduction of thiophene or thiazole moiety to the pyrimidine derivatives might be responsible for antimicrobial activity enhancement of these compounds (Tables 1 and 2).



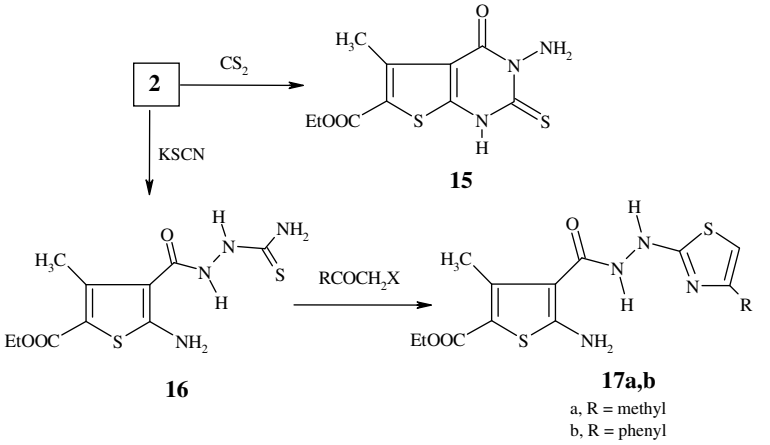
Scheme 1.



Scheme 2.



Scheme 3.



Scheme 4.

Table 1
Antimicrobial activity of the some selected synthesized compounds

Compound	MIC in μg/mL, and zone of inhibition (mm)[a]		
	B. cereus	*S. dysenteriae*	*S. typhi*
2	10	6	—
3a	8	—	8
5	9	12	15
7	33	30	34
9	11	14	11
10	15	7	15
11c	10	—	12
15	19	18	17
16	20	13	28
17a	31	28	29
Ampicillin	21	30	24

—, no inhibition; DMF, negative control.
[a] 1 mg mL^{-1} per disc.

Table 2
Antifungal activity of the some selected synthesized compounds

Compound	MIC in μg/mL, and zone of inhibition (mm)[a]			
	M. phaseolina	*F. equseti*	*A. alternata*	*C. corchori*
2	47.5	29.3	38.8	30.3
3a	34.5	14.8	27.8	29.0
5	46.0	40.0	39.5	33.6
7	95.0	65.6	65.4	54.5
9	49.3	36.3	35.7	34.6
10	48.3	37.5	54.0	50.0
11c	44.2	28.0	27.0	33.2
15	58.6	47.6	42.8	29.0
16	62.0	28.0	34.6	36.4
17a	70.0	43.8	27.0	41.0
Nystatin	71.8	44.7	51.6	40.5

[a] mg mL^{-1} per disc.

Table 3
Acute inflammation in rat using Planimeter for the synthesized compounds

Group	1 h		2 h		3 h		4 h	
	Edema rate (%)	Pot. (%)	Edema rate (%)	Pot. (%)	Edema rate (%)	Pot. (%)	Edema rate (%)	Pot. (%)
Control	89.07 ± 7.46	0	102.78 ± 2.98	0	106.14 ± 3.29	0	122.37 ± 5.83	0
2	77.11 ± 6.48 (−13.43)	37.85	93.69 ± 6.11 (−8.84)	22.22	95.74 ± 5.97 (−9.80)	29.4	106.08 ± 5.41 (−13.32)	32.39
3a	91.37 ± 9.39 (2.58)	−7.27	110.88 ± 0.88 (7.88)	— 19.80	115.11 ± 4.31 (8.46)	— 25.38	122.07 ± 4.46 (−0.25)	0.61
5	65.43 ± 4.01* (−26.54)	74.8	70.88 ± 4.69* (−31.04)	78.01	89.95 ± 4.90 (−20.90)	65.5	89.53 ± 8.32* (−26.84)	65.27
7	43.48 ± 3.78* (−51.18)	144.25	63.52 ± 1.87* (−38.20)	96	74.74 ± 4.88* (−29.58)	88.75	84.05 ± 4.90* (−31.32)	76.17
9	75.68 ± 5.30 (−15.03)	42.36	90.33 ± 6.68 (−12.11)	30.44	101.90 ± 7.85 (−4.00)	0.12	116.01 ± 10.67 (−5.20)	12.65
10	62.51 ± 4.84* (−29.82)	84.05	84.35 ± 3.37 (−17.93)	45.06	90.59 ± 3.75 (−14.65)	43.95	103.73 ± 4.65 (−15.24)	37.06
11c	76.25 ± 4.11 (−14.40)	40.59	101.88 ± 5.16 (−1.52)	3.82	101.99 ± 2.97 (−3.91)	11.73	106.11 ± 3.61 (−13.29)	32.32
15	62.18 ± 4.37* (−30.19)	85.09	87.83 ± 5.03 (−14.54)	36.54	94.91 ± 7.86 (−10.58)	31.74	97.19 ± 9.93* −24.67	59.99
16	56.51 ± 5.40* (36.56)	103.04	64.54 ± 6.36* (−37.21)	93.52	74.93 ± 6.89* (−29.40)	88.21	80.01 ± 7.13* (−34.62)	84.19
17a	48.87 ± 2.49* (−45.14)	127.23	64.58 ± 6.41* (−37.17)	93.42	79.25 ± 3.69* (−25.33)	75.99	89.91 ± 6.01* (−26.53)	64.52
Indomethacin	57.47 ± 3.78* (−35.48)	100	61.88 ± 3.37* (−39.79)	100	70.76 ± 3.25* (−33.33)	100	72.05 ± 3.38* (−41.12)	100

Values represent the mean ± SE of five animals for each group.
Each value in parenthesis indicates the percentage inhibition rate.
The potency (pot.) was calculated compared to the reference drug indomethacin.
* $P < 0.05$: Statistically significant from control using one way ANOVA (Dunnett's as post hoc test).

Table 4
Central analgesic activity (hot plate test)

Group	Basal	30 min	60 min	90 min
Control	8.5 ± 0.30	7.6 ± 0.35	7.1 ± 0.41	6.24 ± 0.39
Aspirin	5.2 ± 0.39	6.66 ± 0.31	6.56 ± 0.52	7.78 ± 0.65
2	5.18 ± 0.50	6.12 ± 0.24	6.20 ± 0.18	7.86 ± 0.68
3a	5.10 ± 0.47	7.72 ± 0.32	7.62 ± 0.35	9.78 ± 0.24*
5	5.85 ± 0.59	10.22 ± 0.35*	12.42 ± 0.70*	10.68 ± 0.22*
7	5.60 ± 0.54	6.08 ± 0.58	6.90 ± 0.38	4.84 ± 0.24*
9	5.54 ± 0.16	7.34 ± 0.17	5.96 ± 0.33	6.08 ± 0.42*
10	5.02 ± 0.27	6.46 ± 0.22	5.04 ± 0.32	6.70 ± 0.39
11c	4.90 ± 0.36	6.42 ± 0.16	7.72 ± 0.68	9.06 ± 0.30
15	4.38 ± 0.40	6.60 ± 0.41	7.24 ± 0.44	7.16 ± 0.31
16	4.80 ± 0.28	5.44 ± 0.42	5.74 ± 0.43	6.14 ± 0.20*
17a	4.80 ± 0.28	6.68 ± 0.33	6.46 ± 0.39	4.70 ± 0.42*

Values represent the mean ± SE of five animals for each groups.
* $P < 0.05$: Statistically significant from control using one way ANOVA (Dunnett's as post hoc test).

The anti-inflammatory activity data (Table 3) indicated that compounds **15**, **10**, **5**, **9**, **11c**, and **2** show significant anti-inflammatory activity in descending order in comparison to the control group, with 85%, 84%, 74%, 42%, 40%, and 37% potency with respect to indomethacin. Compound **3a** have no anti-inflammatory activity. Where compounds **17a**, **16**, and **7** show anti-inflammatory activity in ascending order in comparison to the indomethacin which give higher activity more than the indomethacin with 103%, 127%, and 144% potency.

The results of Analgesic activity (Table 4) indicated that **15**, **10**, **16**, **9**, **7**, and **17a** in descending order, where compounds **5**, **3a**, **11c**, and **2** show higher activity more than Acetyl salicylic acid. Also, the group of rats of compounds **5**, **2**, and **3a** were killed and their stomach were checked for the ulcerogenic effect and show there is no ulcers were found.

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21. All melting points were taken on Electrothermal IA 9100 series digital melting point apparatus. Microanalytical data (in accord with the calculated values) were performed by Vario, Elementar apparatus (Shimadzu). The IR spectra (KBr) were recorded on a Perkin–Elmer 1650 spectrometer (USA). ^{1}H NMR spectra were determined on a JEOL EX-270 run for HNMR at 270 MHz and run

for CNMR at 67.5 MHz; or on JEOL ECA-500 run for HNMR at 500 MHz and run for CNMR at 125 MHz and Chemical shifts were expressed in ppm relative to $SiMe_4$ as internal standards. Mass spectra were recorded on 70 eV EI Ms-QP 1000 EX (Shimadzu, Japan). The Pharmacological evaluations of the products were carried out in Pharmacological Unit, Pharmacology Department, National Research Centre, Dokki, Cairo, Egypt. Biological activity screening was carried out in The Biotechnology and Fermentation Centre, Al-Azhar University, Cairo, Egypt.

3,5-Diethyl-2-amino-4-methylthiophenedicarboxylate (1). According to Gewald et al.[20] and the developed method, a mixture of ethylacetoacetate (0.01 mol), ethylcyano-acetate (0.01 mol), sulfur (0.01 mol) and diethylamine (0.01 mol) was heated (70 °C) under stirring in absolute ethanol for 4 h, then leave the mixture for 24 h at 0 °C. The formed solid was collected by filtration, washed with ethanol (20 mL), dried and crystallized from absolute ethanol, as yellow crystals in a 72% yield, mp 107–109 °C; IR (cm^{-1}, ν); 3420 (br, NH), 3042, 2917 (CH alkyl), 1715, 1718 (2CO); ^1H NMR (DMSO-d_6, δ, ppm): 1.58 (m, 6H, two triplet overlapped, $2CH_3$), 2.49 (s, 3H, CH_3), 4.08 (m, 4H, two quartet overlapped, $2CH_2$), 7.73 (br, 2H, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 257 (M^+); $C_{11}H_{15}NO_4S$ (257.3); Requires (Found): C, 51.34 (51.31); H, 5.87 (5.85); N, 5.44 (5.39).

5-Ethyl-2-amino-3-carbohydrazide-4-methylthiophenecarboxylate (2). A suspension of dry compound **2** (2.57 g, 0.01 mol) in hydrazine hydrate (80%) (5 mL) was stirred under gentle reflux. The insoluble solid dissolved within 10 min with copious evolution of hydrogen sulfide to form a clear solution. After 30 min. when the solid product started separating out, heating was continued for 8 h. The reaction mixture was then allowed to cool to room temperature. The solid was filtered, washed with ethanol, dried and crystallized from dioxane; as yellow crystals in a 80% yield, mp 278–279 °C; IR (cm^{-1}, ν); 3400 (br, NH), 2917 (CH alkyl), 1715, 1690 (2CO); ^1H NMR (DMSO-d_6, δ, ppm): 1.25 (t, 3H, *J* = 7.1 Hz, CH_3), 2.42 (s, 3H, CH_3), 4.18 (q, 2H, *J* = 7.09 Hz, CH_2), 4.35 (br, 2H, NH_2), 7.63 (br, 2H, NH_2), 8.93 (br, 1H, NH) (NH, $2NH_2$, D_2O exchangeable); Its MS (*m*/*z*), 243 (M^+); $C_9H_{13}N_3O_3S$ (243.3); Requires (Found): C, 44.43 (44.39); H, 5.38 (5.37); N, 17.27 (17.29).

5-Ethyl-2-amino-3-[(3,5-dimethylpyrazolyl derivatives)-carbonyl]-4-methylthiophene-carboxylate (3a, b). General procedure. A mixture of compound **2** (0.01 mol) and the β-diketone (0.01 mol) in absolute ethanol (30 mL) was stirred under reflux for 12 h. The reaction mixture was allowed to cool to 0 °C for 24 h, the solid precipitate was filtered off, dried and crystallized from an appropriate solvent to produce **3a**, **b** in high yields.

5-Ethyl-2-amino-3-[(3,5-dimethylpyrazol-1-yl)-carbonyl]-4-methylthiophene-carboxylate (3a). It was obtained from the reaction of **2** (0.01 mol) with pentan-2,4-dione (0.01 mol), as yellow crystals, crystallized from ethanol in a 80% yield, mp 152–153 °C; IR (cm^{-1}, ν); 3380 (br, NH), 2917 (CH alkyl), 1710, 1690 (2CO), 1625 (C=N), 1540 (C=C); ^1H NMR (DMSO-d_6, δ, ppm): 1.30 (t, 3H, *J* = 7.0 Hz, CH_3), 2.19 (s, 3H CH_3), 2.51 (s, 3H, CH_3), 2.67 (s, 3H, CH_3), 4.25 (q, 2H, *J* = 7.10 Hz, CH_2), 6.21 (s, 1H, pyrazole proton), 7.98 (br, 2H, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 308 (M^+, 12%), 307 (M^+-1, 57%), 212 (M^+-$C_5H_8N_2$, 100%); $C_{14}H_{18}N_3O_3S$ (308.4); Requires (Found): C, 54.52 (54.49); H, 5.88 (5.86); N, 13.62 (13.59).

5-Ethyl-2-amino-3-[(4-chloro-3,5-dimethylpyrazol-1-yl)-carbonyl]-4-methyl-thiophene-carboxylate (3b). It was obtained from the reaction of **2** with 3-chloropentan-2,4-dione (0.01 mol), as a light yellow powder and crystallized from ethanol in a 76% yield, mp 103–105 °C; IR (cm^{-1}, ν); 3400 (br, NH), 2929 (CH alkyl), 1712, 1688 (2CO), 1620 (C=N), 1550 (C=C); ^1H NMR (DMSO-d_6, δ, ppm): 1.32 (t, 3H, *J* = 7.1 Hz, CH_3), 2.21 (s 3H CH_3), 2.52 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 4.22 (q, 2H, *J* = 7.08 Hz, CH_2), 8.02 (br, 2H, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 342 (M^+ + 1, 28%), 341 (M^+, 56%), 212 (M^+-$C_5H_7ClN_2$, 100%); $C_{14}H_{16}ClN_3O_3S$ (341.8); Requires (Found): C, 49.19 (49.17); H, 4.72 (4.71); N, 12.29 (12.26).

5-Ethyl-2-amino-3-[(3-methyl-5-oxo-4,5-dihydropyrazol-1-yl)carbonyl]-4-methyl-thiophenecarboxylate (4). A solution of compound **2** (0.01 mol) and ethylacetoacetate (0.01 mol) in sodium ethoxide solution (prepared by dissolving (0.01 mol) of sodium metal in (30 mL) absolute ethanol was heated under reflux with stirring for 6 h. The reaction mixture was allowed to cool and poured onto cold water (100 mL) and neutralized by acetic acid, whereby a solid was precipitated, which was filtered off and crystallized from ethanol, as a yellow powder in a 74% yield, mp 150–152 °C; IR (cm^{-1}, ν); 3410 (br, NH), 2936 (CH alkyl), 1705, 1697, 1684 (3CO), 1550 (C=N), 1500 (C=C); ^1H NMR (DMSO-d_6, δ, ppm): 1.31 (t, 3H, *J* = 7.2 Hz, CH_3), 2.23 (s, 3H, CH_3), 2.52 (s, 2H, CH_3), 2.86 (s, 2H, CH_2), 4.23 (q, 2H, *J* = 7.09 Hz, CH_2), 8.00 (br, 2H, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 309 (M^+); $C_{13}H_{15}N_3O_4S$ (309.3); Requires (Found): C, 50.47 (50.49); H, 4.88 (4.86); N, 13.58 (13.55).

5-Ethyl-2-amino-3-[(3,5-dioxopyrazolidin-1-yl)carbonyl]-4-methylthiophene-carboxylate (5). A solution of compound **2** (0.01 mol) and freshly distilled diethylmalonate (0.01 mol) in sodium ethoxide solution (prepared by dissolving (0.01 mol) of sodium metal in (30 mL) absolute ethanol was heated under reflux with stirring for 5 h. The solvent was evaporated under reduced pressure, and the crude was acidified with 10% hydrochloric acid. The solid formed was filtered off, washed with cold water, and crystallized from ethanol, as yellow powder in 70% yield, mp 212–214 °C; IR (cm^{-1}, ν); 3400 (br, NH), 2928 (CH alkyl), 1700, 1690, 1684, 1678 (4CO), 1580 (C=N), 1520 (C=C); ^1H NMR (DMSO-d_6, δ, ppm): 1.32 (t, 3H, *J* = 7.0 Hz, CH_3), 2.23 (s, 3H, CH_2), 2.80 (s, 2H, CH_2), 4.25 (q, 2H, *J* = 7.11 Hz, CH_2), 8.06 (br, 2H, NH_2, D_2O exchangeable), 8.86 (br, NH, D_2O exchangeable); Its MS (*m*/*z*), 311 (M^+); $C_{12}H_{13}N_3O_5S$ (311.3); Requires (Found): C, 46.29 (46.27); H, 4.20 (4.18); N, 13.49 (13.46).

5-Ethyl-2-amino-3-(N-ethoxymethylene-carbohydrazide)-4-methylthiophene-carboxylate (6). A solution of compound **2** (0.01 mol) and triethylorthoformate (20 mL) was heated under reflux with stirring for 2 h. The reaction mixture was filtered while hot and then cooled to rt. The solid formed was filtered off, dried and crystallized from ethanol, as yellow powder in 78% yield, mp 182–184 °C: IR (cm^{-1}, ν); 3390 (br s, NH), 2929 (CH alkyl), 1700, 1685, (2CO), 1572 (C=N), 1509 (C=C): ^1H NMR (DMSO-d_6, δ, ppm): 1.21 (t, 3H, *J* = 14.3 Hz, CH_3), 1.35 (t, 3H, *J* = 7.03 Hz, CH_3) 2.24 (s, 3H, CH_2), 4.18 (q, 2H, *J* = 7.13 Hz, CH_2), 4.28 (q, 2H, *J* = 7.08 Hz, CH_2), 6.88 (s, 1H, azomethine proton), 8.50 (br, 2H, NH_2, D_2O exchangeable), 10.02 (br, NH, D_2O exchangeable); Its MS (*m*/*z*), 299 (M^+); $C_{12}H_{17}N_3O_4S$ (299.3); Requires (Found): C, 48.14 (48.11); H, 5.72 (5.69); N, 14.03 (13.98).

5-Ethyl-2-amino-3-(N-hydroxymethylene-carbohydrazide)-4-methylthiophene-carboxylate (7). A mixture of **6c** (0.01 mol) and formic acid (10 mL), was heated under reflux for 6 h. The reaction mixture was allowed to cool to room temperature and poured onto water (100 mL). The solid formed was collected by filtration, dried, and crystallized from benzene, orange powder, in 80% yield, mp 198–2001 °C; IR (cm^{-1}, ν): 3415 (br, NH), 2920 (CH alkyl),1724, 1715, 1680 (3CO), 1560 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.33 (t, 3H, *J* = 7.03 Hz, CH_3), 2.52 (s, 3H, CH_2), 4.35 (q, 2H, *J* = 7.09 Hz, CH_2), 8.05 (br, 2H, NH_2, D_2O exchangeable), 8.65 (br, NH, D_2O exchangeable), 9.02 (s, 1H, aldehydic proton), 11.30 (br, OH, D_2O exchangeable); Its MS (*m*/*z*), 271.3 (M^+); $C_{10}H_{13}N_3O_4S$ (507.5); Requires (Found): C, 44.27 (44.24); H, 4.83 (4.81); N, 15.49 (15.51).

5-Ethyl-2-amino-3-(1,3,4-oxa/thiadiazol-2-yl)-4-methylthiophenecarboxylate (8a, b). General Procedure. A mixture of compound **7** (0.005 mol) and phosphorus pentaoxide or phosphorus pentasulfide (4.00 g) was heated under reflux for 12 h, in dry xylene (30 mL). The solid that separated upon cooling was filtered off, crystallized from appropriate solvent to produce (**8a**, **b**) in good yield.

5-Ethyl-2-amino-3-(1,3,4-oxadiazol-2-yl)-4-methyltiophenecarboxylate (8a). Method A. It was obtained from the reaction of **7** with phosphorus pentoxide, as yellow powder and crystallized from ethanol/dioxane (1:1) in 76% yield, mp 211–213 °C; IR (cm^{-1}, ν): 3385 (br, NH), 3026 (CH aryl), 2920 (CH alkyl), 1712 (CO), 1560 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.30 (t, 3H, *J* = 7.01 Hz, CH_3), 2.54 (s, 3H, CH_2), 4.28 (q, 2H, *J* = 7.11 Hz, CH_2),7.98 (s, CH, oxadiazole proton), 8.25 (br, 2H, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 253 (M^+); $C_{10}H_{11}N_3O_3S$ (253.2); Requires (Found): C, 47.42 (47.39); H, 4.37 (4.39); N, 16.59 (16.62).**Method B**. Compound 6 (0.005 mol) was heated at 210 °C for 30 min. The reaction product was purified preparative TLC on silica gel using chloroform/ethylacetate (80:20) as an eluent to give **8a**.

5-Ethyl-2-amino-3-(1,3,4-thiadiazol-2-yl)-4-methyltiophenecarboxylate (8b). It was obtained from the reaction of **7** with phosphorus pentasulfide, as yellow powder and crystallized from ethanol/dioxane (1:1) in a 78% yield, mp 230–231 °C; IR (cm^{-1}, ν): 3392 (br, NH), 3029 (CH aryl), 2918 (CH alkyl), 1718 (CO), 1568 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.32 (t, 3H, *J* = 7.02 Hz, CH_3), 2.51 (s, 3H, CH_2), 4.30 (q, 2H, *J* = 7.09 Hz, CH_2), 8.05 (s, CH, thiadiazole proton), 8.40 (br, 2H, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 269 (M^+, 100%); $C_{10}H_{11}N_3O_2S_2$ (269.3); Requires (Found): C, 44.59 (44.57); H, 4.11 (4.09); N, 15.60 (15.57).

3,5-Diethyl-2-acetylamino-4-methylthiophenedicarboxylate (9). It was obtained from the reaction of **1** (0.01 mol) with acetic anhydride (30 mL) was heated under reflux for 3 h. The reaction mixture was allowed to cool to room temperature and the solid formed was collected by filtration, dried, and crystallized from ethanol, as yellow powder and crystallized from ethanol in a 90% yield, mp 129–130 °C; IR (cm^{-1}, ν): 3400 (br, NH), 2920 (CH alkyl), 1718, 1685 (2CO), 1530 (C=C); ^1H NMR (DMSO-d_6, δ, ppm): 1.29 (t, 3H, *J* = 7.02 Hz, CH_3), 1.35 (t, 3H, *J* = 14.00 Hz, CH_3), 2.24 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 4.26 (q, 2H, *J* = 7.08 Hz, CH_2), 4.36 (q, 2H, *J* = 7.10 Hz, CH_2), 11.10 (br, NH, D_2O exchangeable); Its MS (*m*/*z*), 299 (M^+, 100%); $C_{13}H_{17}NO_5S$ (299.3); Requires (Found): C, 52.15 (52.17); H, 5.72 (5.74); N, 4.67 (4.59).

6-Carboxyethyl-2,5-dimethyl-3-(N-amino)-thieno[2,3-d]pyrimidin-4-one (10). It was obtained from the reaction of **9** (2.99 g, 0.01 mol) with hydrazine hydrate (10 mL, 80%) in ethanol (30 mL) was heated under reflux for 2 h. The reaction mixture was allowed to cool to room temperature and poured onto water (100 mL). The solid formed was collected by filtration, dried and crystallized from ethanol, as yellow powder and crystallized from ethanol in a 83% yield, mp 205–207 °C; IR (cm^{-1}, ν): 3405 (br, NH), 2920 (CH alkyl), 1715, 1683 (2CO), 1570 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.34 (t, 3H, *J* = 7.01 Hz, CH_3), 2.29 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 4.35 (q, 2H, *J* = 7.12 Hz, CH_2), 8.56 (br, 2H, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 267, (M^+, 100%); $C_{11}H_{13}N_3O_3S$ (267.3); Requires (Found): C, 49.42 (49.39); H, 4.90 (4.93); N, 15.72 (15.69).

5-Ethyl-2-amino-3-(arylmethylenehydrazide)-4-methylthiophene-carboxylate (11a–c). General procedure. A mixture from compound **2** (2.43 g, 0.01 mol) and the appropriate aromatic aldehyde (0.01 mol) was stirred under reflux in glacial acetic acid (10 mL) for 10 min. The reaction mixture was allowed to cool to room temperature; the solid separated was filtered off and crystallized from appropriate solvent to produces **11a–c** in high yields.

5-Ethyl-2-amino-3-(phenylmethylenehydrazide)-4-methylthiophenecarboxylate (11a). It was obtained from **2** and benzaldehyde (1.06 g), as yellow crystals and crystallized from ethanol in a 83% yield, mp 120–123 °C; IR (cm^{-1}, ν); 3388 (br, NH), 3038 (CH aryl), 2925 (CH alkyl), 1695, 1688 (2CO), 1625 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.27 (t, 3H, *J* = 7.0 Hz, CH_3), 2.43 (s, 3H, CH_3), 4.16 (q, 2H, *J* = 7.06 Hz, CH_2), 7.14–7.29 (m, 5H, Ar-H), 7.90 (br, 2H, NH_2), 8.12 (s, 1H, azomethine proton), 8.93 (br, 1H, NH) (NH, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 331 (M^+, 100%); $C_{16}H_{17}N_3O_3S$ (331.4); Requires (Found): C, 57.98 (57.96); H, 5.17 (5.68); N, 12.68 (12.66).

5-Ethyl-2-amino-3-(4-chlorophenylmethylenehydrazide)-4-methylthiophenecarboxylate (11b). It was obtained from **2** and 4-chlorobenzaldehyde (1.40 g), as yellow crystals and crystallized from dioxane in a 86% yield, mp 195–197 °C; IR (cm^{-1}, ν); 3400 (br, NH), 3040 (CH aryl), 2929 (CH alkyl), 1700, 1686 (2CO), 1630 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.24 (t, 3H, *J* = 7.0 Hz, CH_3), 2.41 (s, 3H, CH_3), 4.19 (q, 2H, *J* = 7.05 Hz, CH_2), 7.14 (d, 2H, *J* = 8.02 Hz, Ar-H),7.69 (d, 2H, *J* = 8.02 Hz, Ar-H), 8.02 (br, 2H, NH_2), 8.10 (s, 1 H, azomethine proton), 9.10 (br, 1H, NH) (NH, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 366 (M^+ + 1, 28%), 365 (M^+, 100%); $C_{16}H_{16}ClN_3O_3S$ (365.8); Requires (Found): C, 52.52 (52.49); H, 4.41 (4.39); N, 11.48 (11.50)..

5-Ethyl-2-amino-3-(4-methoxyphenylmethylenehydrazide)-4-methylthiophenecarboxylate (11c). It was obtained from **2** and 4-methoxybenzaldehyde (1.36 g), as yellow powder, crystallized from dioxane in a 81% yield, mp 166–167 °C; IR (cm^{-1}, ν); 3396 (br, NH), 3025 (CH aryl), 2934 (CH alkyl), 1705, 1687 (2CO), 1635 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.25 (t, 3H, *J* = 7.02 Hz, CH_3), 2.43 (s, 3H, CH_3), 4.17 (q, 2H, *J* = 7.08 Hz, CH_2), 4.34 (s, 3H, OCH_3), 7.25 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.70 (d, 2H, *J* = 8.01 Hz, Ar-H), 7.98 (br, 2H, NH_2), 8.18 (s, 1H, azomethine proton), 9.40 (br, 1H, NH) (NH, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 361 (M^+, 100%); $C_{17}H_{19}N_3O_4S$ (361.4); Requires (Found): C, 56.49 (56.47); H, 5.29 (5.26); N, 11.62 (11.56).

5-Ethyl-2-acetamido-3-(arylmethylenehydrazide)-4-methylthiophenecarboxylate (13a–c). General procedure. A solution of each of compound **11a–c** (0.01 mol) in 30 mL acetic anhydride was stirred under reflux for 4 h (TLC). The excess of the solvent was evaporated under vacuum to dryness. The solid product that formed was crystalized from appropriate solvent to produces **13a–c** in high yields.

5-Ethyl-2-acetamido-3-(phenylmethylenehydrazide)-4-methylthiophenecarboxylate (13a). It was obtained from **11a**, as yellow crystals and crystallized from ethanol in 78% yield, mp 190–193 °C; IR (cm^{-1}, ν); 3386 (br, NH), 3032 (CH aryl), 2920 (CH alkyl), 1696, 1685, 1680 (3CO), 1615 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.23 (t, 3H, *J* = 7.01 Hz, CH_3), 2.45 (s, 3H, CH_3), 3.02 (s, 3H, CH_3), 4.13 (q, 2H, *J* = 7.06 Hz, CH_2), 7.18–7.34 (m, 5H, Ar-H), 8.08 (s, 1H, azomethine proton), 8.95, 9.80 (2br, 2H, 2NH, D_2O exchangeable); Its MS (*m*/*z*), 373 (M^+, 100%); $C_{18}H_{19}N_3O_4S$ (373.4); Requires (Found): C, 57.89 (57.90); H, 5.12 (5.09); N, 11.25 (11.26).

5-Ethyl-2-acetamido-3-(4-chlorophenylmethylenehydrazide)-4-methylthiophenecarboxylate (13b). It was obtained from **11b**, as yellow crystals and crystallized from dioxane in 70% yield, mp 213–215 °C; IR (cm^{-1}, ν); 3410 (br, NH), 3025 (CH aryl), 2932 (CH alkyl), 1705, 1688, 1680 (3CO), 1615 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.22 (t, 3H, *J* = 7.02 Hz, CH_3), 2.40 (s, 3H, CH_3), 3.05 (s, 3H, CH_3), 4.16 (q, 2H, *J* = 7.06 Hz, CH_2), 7.22 (d, 2H, *J* = 8.01 Hz, Ar-H),7.75 (d, 2H, *J* = 8.01 Hz, Ar-H), 8.12 (s, 1H, azomethine proton), 9.20, 10.00 (2br, 2H, 2NH, D_2O exchangeable); Its MS (*m*/*z*), 408 (M^+ + 1, 31%), 407 (M^+, 100%); $C_{18}H_{18}ClN_3O_4S$ (407.8); Requires (Found): C, 53.00 (53.02); H, 4.44 (4.39); N, 10.30 (10.34).

5-Ethyl-2-acetamido-3-(4-methoxyphenylmethylenehydrazide)-4-methylthiophenecarboxylate (13c). It was obtained from **11c**, as yellow powder, crystallized from ethanol in 73% yield, mp 231–233 °C; IR (cm^{-1}, ν); 3400 (br, NH), 3034 (CH aryl), 2918 (CH alkyl), 1695, 1688, 1675 (3CO), 1620 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.26 (t, 3H, *J* = 7.0 Hz, CH_3), 2.44 (s, 3H, CH_3), 3.01 (s, 3H, CH_3), 4.20 (q, 2H, *J* = 7.06 Hz, CH_2), 4.30 (s, 3H, OCH_3), 7.28 (d, 2H, *J* = 8.04 Hz, Ar-H), 7.78 (d, 2H, *J* = 8.04 Hz, Ar-H), 8.11 (s, 1H, azomethine proton), 9.00, 9.40 (2br, 2H, 2NH, D_2O exchangeable); Its MS (*m*/*z*), 403 (M^+, 100%); $C_{19}H_{21}N_3O_5S$ (403.4); Requires (Found): C, 56.56 (56.53); H, 5.24 (5.22); N, 10.41 (10.39).

2,5-Dimethyl-5-carboxyethyl-thieno[2,3-d]pyrimidin-4(H)-one (14). General procedure. A solution of each of compound **13a–c** (0.01 mol) in an ethanolic sodium ethoxide solution (prepared by dissolving 0.23 g of sodium metal in 30 mL ethanol), was stirred under reflux for 6 h (TLC). The excess of the solvent was evaporated under vacuum to dryness. The solid product that formed was crystalized from dimethylformamide, as yellow crystals and crystallized from dimethylformamide in 72% yield, mp 267–270 °C; IR (cm^{-1}, ν); 3385 (br, NH), 2918 (CH alkyl), 1700, 1687 (2CO), 1640 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.30 (t, 3H, *J* = 7.03 Hz, CH_3), 2.40 (s, 3H, CH_3), 2.79 (s, 3H, CH_3), 4.21 (q, 2H, *J* = 7.06 Hz, CH_2), 9.30 (br, 1H, NH, D_2O exchangeable); Its MS (*m*/*z*), 252 (M^+, 100%); $C_{11}H_{12}N_2O_3S$ (252.2); Requires (Found): C, 52.36 (52.29); H, 4.79 (4.76); N, 11.09 (11.12).

3-Amino-6-carboxyethyl-5-methyl-2-thioxo-thieno[2,3-d]pyrimidin-4-one (15). To a warmed ethanolic sodium hydroxide solution (0.40 g in 50 mL ethanol), compound **2** (2.43 g, 0.01 mol), and carbon disulfide (excess 5 mL) were added. The mixture was heated under reflux for 15 h. The reaction mixture was allowed to cool to 0 °C, the deposited precipitate was filtered off, washed by water (20 mL), dried, and crystallized from ethanol as brown crystals; in 68% yield, mp 192–194 °C; IR (cm^{-1}, ν); 3430 (br, NH), 2923 (CH alkyl), 1690, 1675 (2CO); ^1H NMR (DMSO-d_6, δ, ppm): 1.28 (t, 3H, *J* = 7.00 Hz, CH_3), 2.44 (s, 3H, CH_3), 4.19 (q, 2H, *J* = 7.04 Hz, CH_2), 7.54 (br, 2H, NH_2), 9.30 (br, 1H, NH) (NH, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 285 (M^+, 100%); $C_{10}H_{11}N_3O_3S_2$ (285.3); Requires (Found): C, 42.09 (42.07); H, 3.88 (3.85); N, 14.72 (14.76).

5-Ethyl-2-amino-3-carbonylthiosemicarbazide-4-methylthiophenecarboxylate (16). A mixture of **2** (0.01 mol) was refluxed with 10 mL of 10% HCl and potassium thiocyanate (0.015 mol) for 4 h. The reaction mixture was allowed to cool to room temperature. The solid formed was collected by filtration, washed with water, dried and then crystallized from dioxane/DMF (2:1), as a yellow powder in a 78% yield, mp 226–227 °C; IR (cm^{-1}, ν); 3400 (br, NH), 2915 (CH alkyl), 1690, 1678 (2CO); ^1H NMR (DMSO-d_6, δ, ppm): 1.27 (t, 3H, *J* = 7.00 Hz, CH_3), 2.45 (s, 3H, CH_3), 4.16 (q, 2H, *J* = 7.05 Hz, CH_2), 5.30 (br, 2 H, NH_2), 7.60 (br, 2H, NH_2), 8.95, 9.30 (2br, 2H, 2NH) (2NH, $2NH_2$, D_2O exchangeable); Its MS (*m*/*z*), 302 (M^+, 100%); $C_{10}H_{14}N_4O_3S_2$ (302.3); Requires (Found): C, 39.72 (39.67); H, 4.66 (4.64); N, 18.53 (18.46).

5-Ethyl-2-amino-3(4-methyl/or phenyl-1,3-thiazol-2-yl)carbohydrazide-4-methylthiophenecarboxylate (17a, b), General procedure; A mixture of compound **16** (0.01 mol) and chloroacetone or phenacylbromide (0.01 mol) was refluxed in ethanol (50 mL) for 8–10 h. The reaction mixture was then kept overnight, the solid that separated was filtered off and crystallized from *n*-hexane to produce (**17a**, **b**) in high yield .

5-Ethyl-2-amino-3(4-methyl-1,3-thiazol-2-yl)carbohydrazide-4-methylthiophenecarboxylate (17a). It was obtained from compound **16** and chloroacetone; as white crystals, crystallized from *n*-hexane in 66% yield, mp 134–135 °C (dec): IR (cm^{-1}, ν); 3425 (br, NH), 2927 (CH alkyl), 1692, 1675 (2CO), 1635 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.23 (t, 3H, *J* = 7.01 Hz, CH_3),2.22 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 4.21 (q, 2H, *J* = 7.03 Hz, CH_2), 7.90 (br, 2H, NH_2), 8.16 (s, CH, thiazole proton), 9.05, 10.20 (2br, 2H, 2NH) (2NH, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 340 (M^+, 70%); $C_{13}H_{16}N_4O_3S_2$ (340.4); Requires (Found): C, 45.86 (45.83); H, 4.73 (4.69); N, 16.46 (16.42).

5-Ethyl-2-amino-3(4-phenyl-1,3-thiazol-2-yl)carbohydrazide-4-methylthiophenecarboxylate (17b). It was obtained from compound **16** and phenacylbromide, as a yellow powder, crystallized from benzene in 53% yield, mp 107–109 °C (melted); 3430 (br, NH),3035 (CH, aryl), 2915 (CH alkyl), 1689, 1670 (2CO), 1620 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.28 (t, 3H, *J* = 7.02 Hz, CH_3), 2.47 (s, 3H, CH_3), 4.19 (q, 2H, *J* = 7.05 Hz, CH_2), 7.09–7.23 (m, 5H, Ar-H), 8.05 (br, 2H, NH_2), 8.20 (s, CH, thiazole proton), 9.00, 10.15 (2br, 2H, 2NH) (2NH, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 402 (M^+, 43%); $C_{18}H_{18}N_4O_3S_2$ (402.4); Requires (Found): C, 53.71 (53.69); H, 4.50 (4.47); N, 13.92 (13.89).

Antibacterial activity. The newly synthesized compounds were screened for their antibacterial activity against pathogenic organisms; *Bacillus cereus* (BTCC19), *Shigella dysenteriae* (AE 14396), and *Salmonella typhi* (AE 14612) (Table 1) and for antifungal activity against *Macrophomina phaseolina* (Tassi) Goid, *Fusarium equiseti* (Corda) Sacc, *Alternaria alternata* (Fr.) Kedisslar and *Coillectotrium corchori* Ikata (Yoshida) (Table 2). The disc diffusion method[22] and poisoned-food techniques[23] were used for antibacterial and antifungal activities, respectively. The tested compounds were dissolved in *N*,*N*-dimethylformamide (DMF) to get a solution of 1 mg mL^{-1}. The inhibition zones were measured in millimeters at the end of an incubation period of 48 h at 28 °C. DMF alone showed no inhibition zone. Nutrient agar (NA) and potato dextrose agar (PDA) were used as basal media to test the bacteria and fungi, respectively. Commercial antibacterial ampicillin and antifungal nystatin were also tested under similar conditions for comparison.

Anti-inflammatory activity (Carrageenin induced rat hind paw edema model). The method adopted resembles essentially that described by Winter et al.[24] (0.5 mL of tween-80 in distilled water) was selected as vehicle to suspend the standard drugs and the test compounds. The albino rats weighing between 150 and 180 g was starved for 18 h prior to the experiment. The animals were weighed, marked for identification and divided into 12 groups each group containing five animals. Edema was induced in the left hind paw of all rats by subcutaneous injection of 0.1 mL of 1% (W/V) carrageenin in distilled water into their fotpads. The 1st group was kept as control and was given the respective volume of the solvent (0.5 mL of tween-80 in distilled water). The 2nd to 11th groups were orally administered aqueous suspension of the synthesized compounds in dose of (15 mg/kg body weight) 1 h before carrageenin injection. The last group (standard) was administered indomethacin in a dose of 10 mg/kg body weight, orally as aqueous suspension.[25] The paw volume of each rat was measured immediately by mercury plethysmometer, before carrageenin injection and then hourly for 4 h post administration of aqueous suspension of the synthesized compounds. The edema rate and inhibition rate of each group were calculated as follows, (Edema rate (*E*) % = $V_t - V_o/V_o$, Inhibition rate (*I*) % = $E_c - E_t/E_c$) where V_t is the volume before carrageenin injection (mL), Vt is the volume at *t* hours after carrageenin injection (mL) E_c, E_t the edema rat of control group and treated group, respectively. Analgesic activity using hot plate test. The experiment was carried out as described by Turner[26] using hot plate apparatus, maintained at 53 ± 0.5 °C. The mice were divided into 12 groups of five animals each. The reaction time of the albino mice to the thermal stimulus was the time interval between placing the animal in the hot plate and when it licked its hind paw or jumped. Reaction time was measured prior to aqueous suspension of synthesized compounds and drug treatment (0 min). Group 1 was kept as normal control. The aqueous suspension of synthesized compounds was orally administered to mice of groups 2 to 11 at doses of 20 mg/kg. Mice of group 12 (reference) were orally treated with aspirin in a dose of 20 mg/kg b.w. The reaction time was again measured at 15 min and repeated at 0, 30, 60, and 90 min. after treatment. To avoid tissue damage to the mice paws, cut-off time for the response to the thermal stimulus was set at 60 s. The reaction time was calculated for each synthesized compounds and drug-treated group.

Animals. Both sex of albino rats (150–200 g) were used in the study of anti-inflammatory activity and both sex of Swiss albino mice weighing (25–30 g) used in analgesic activity and, taking into account international principle and local regulations concerning the care and used of laboratory animals.[27] The animals had free access to standard commercial diet and water ad libitum and were kept in rooms maintained at 22 ± 1 °C with 12 h light dark cycle.

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