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Graphic

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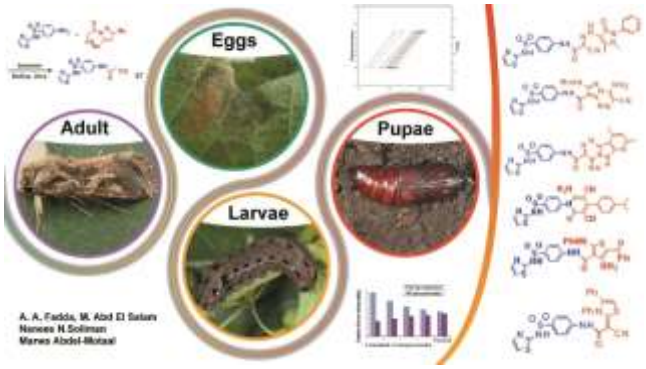

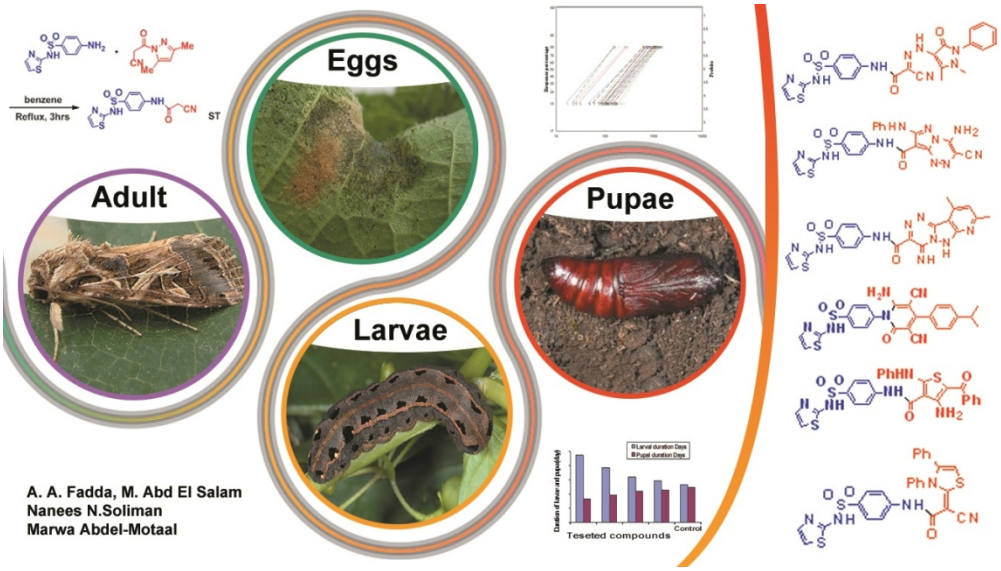
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# Synthesis, Characterization and Biochemical impacts of some new bioactive sulfonamide thiazole derivatives as potential insecticidal agents against the cotton leafworm, *Spodoptera littoralis*

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## ABSTRACT

A novel series of anticipated biologically active heterocyclic compounds such as pyrazole, thiazole, pyridine, acrylamide, thiophene, triazolo[1,5-a]pyrimidine, imidazolidine, aminopyrazole, pyrazolo[5,1-c][1,2,4]triazine, triazolo[4,3-a]pyrimidine, benzo[4,5]imidazo[1,2-a]pyrimidine, pyrido[2',3':3,4]pyrazolo[5,1-c][1,2,4]triazine, isoxazole, benzo[4,5]imidazo[2,1-c][1,2,4]triazine, pyrimidine, pyrido[2',3':3,4]pyrazolo[1,5-a]pyrimidine, pyrano[2,3-d]pyrimidine and chromene derivatives incorporating Sulfonamide-bearing thiazole moiety suitable for utilize as insecticidal agents were synthesized *via* a versatile, readily accessible cyanoacetanilide, 2-cyano-*N*-(4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl)acetamide **(1)**.The structures of the newly synthesized compounds were elucidated by IR, MS, $^{1}$H NMR, $^{13}$C NMR, DEPT, H-H COSY, HMBC, and HSQC spectral analysis. Toxicological, biochemical parameters and biological aspects of the demonstrative compounds of the synthesized products against the cotton leafworm, *Spodoptera littoralis* under laboratory conditions were also investigated. Regarding the determined $LC_{50}$ and $LC_{90}$ values, sulfonamides bearing thiazole moiety **16a**, **8**, **28** and **31b** showed the most potent toxic effects with $LC_{50}$ values of 49.04, 62.66, 78.62, 94.90 and 105.10 ppm, respectively, and toxicity index being 100, 78.26, 62.38, 51.68 and 46.66%, respectively.


**KEYWORDS:** Sulfonamide; Cyanoacetanilide; Thiazole; Insecticidal activity; Biochemical parameters; Biological aspects; *Spodoptera littoralis*.

## INTRODUCTION

Recently, sulfur-containing pesticidal chemical families namely sulfonamides have a great interest in modulating the properties of new crop protection compounds, mainly in fungicides, herbicides and insecticides. As the modern agricultural chemistry has to support farmers by providing innovative agrochemicals[1]. On the other hand, sulfonamides exhibit a broad spectrum of biological activities including antibacterial[2], carbonic anhydrase inhibitory functions[3,4], insulin release inducer[5], antiviral[6], antifungal[7], anticancer[8] and anti-inflammatory activities[9]. It is known that sulfonamides reduce the biosynthesis of dihydrofolic acid through the competitive inhibition of the dihydropteroate synthase enzyme (DHPS) which prevents the growth and reproduction of microorganisms[10]. Moreover, a sulfonamide is versatile moiety for its diverse pharmacological activities that include antitumor[11], anticonvulsant[12].Acetazolamide (AAZ), dorzalamide (DZA) and brinzolamide (BRZ) are sulfonamide derivatives and used in the treatment of glaucoma[13–15].

Cyanoacetanilide derivatives are versatile and important reagents, which have especially been utilized as building blocks for the synthesis of polyfunctionalized heterocyclic compounds with different ring sizes and condensed heterocycles that possess a broad spectrum of biological activities[16]. Cyanoacetanilides are polyfunctional compounds that exhibit both electrophilic and nucleophilic aspects[17]. Two nucleophilic centers in cyanoactanilides are localized on NH and methylene group. Also, cyanoacetanilides possess two electrophilic positions[18], which are associated with C=O and CN. So, region selective attack on the cyanoacetanilide moiety of the precursor **1** by

different reagents was reported herein for the synthesis of versatile, highly functionalized heterocyclic compounds.

From these findings, it was thought worthwhile to build up some innovative bioactive polyfunctionallized substituted heterocyclic compounds incorporating a sulfonamide thiazole moiety of potential insecticidal efficacy against the cotton leafworm, *Spodoptera littoralis* under laboratory conditions. Moreover, estimation of the mode of action of the most potent tested insecticides by determination of biochemical parameters, (Enzymatic activity) such as Alk-p, ALT, AST, T. protein and Ach-E, in addition to, histological studies. Furthermore, assessment of the latent effects of the tested compounds on some biological aspects such as larval and pupal duration, pupal weight, percentage of normal, deformed pupae and adult emergency, percentage of fecundity and egg hatchability, moreover, adult longevity with a hope to get better insecticidal agents slightly side effects.

## EXPERIMENTAL

All spectroscopic analysis were carried out as mentioned in the reported work[19].

### Synthesis of the cyanoacetanilide 2-cyano-N-(4-(N-(thiazol-2 yl)sulfamoyl)phenyl)acetamide (1)

To a (25 mL) dry benzene solution, equimolar amounts of sulfathiazole (5.10 g, 0.02 mol), and 3-(3,5-dimethyl-1H-pyrazol-1-yl)-3-oxopropanenitrile (3.26 g, 0.02 mol) were supplemented, and the mixture was heated under reflux for 3 h. A solid precipitate was obtained after cooling to room temperature was filtered and recrystallization from ethanol to give **1**. White powder; mp 245-247°C; yield 92%; IR (KBr) ν/cm-1: 3459, 3366 (2NH), 2258 (CN), 1713 (CO); $^{1}$H-NMR (400 MHz, DMSO-d6): δH ppm 3.95 (s, 2H, $CH_2$), 6.82

(d, 1H, thiazole H-5, J= 4.4 Hz), 7.25 (d, 1H, thiazole H-4, J= 4.4 Hz), 7.68 (d, 2H, Ar-H, J= 8.8 Hz), 7.77 (d, 2H, Ar-H, J= 8.8 Hz), 10.62 (s, 1H, NHCO), 12.70 (s, 1H, $NHSO_2$). MS m/z (%): 323 (M+ + 1, 1.24), 322 (M+, 3.36), 282 (1.05), 281 (5.42), 258 (27.11), 257 (23.84), 255 (26.23), 239 (2.68), 234 (3.76), 223 (8.63), 216 (8.35), 192 (15.46), 191 (100), 190 (19.08), 167 (10.04), 159 (10.25), 156 (55.45), 140 (13.23), 118 (4.09), 108 (43.55), 93 (15.48), 92 (57.58), 91 (7.66), 65 (28.59), 55 (12.50), 45 (9.04). Anal. for $C_{12}H_{10}N_4O_3S_2$ (322.36): calcd.: C, 44.71; H, 3.13; N, 17.38%; found: C, 44.58; H, 3.09; N, 17.35%.

**Synthesis of Z)-3-amino-3-(hydroxyimino)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)propanamide (2)**

Compound **1** (0.4 g, 0.0012 mol) and hydroxylamine hydrochloride (0.08 g, 0.0012 mol) was refluxed in boiling EtOH (25 mL) with few drops of TEA (3 drops) for 3 h. The obtained solid material on cooling was filtered and recrystallized from ethanol to give compound **2**. White powder; mp 275–277 °C; yield 75%; IR (KBr) ν/cm-1: 3512 (OH), 3446 (2NH), 3341, 3300 ($NH_2$), 1677 (CO). Anal. for $C_{12}H_{13}N_5O_4S_2$ (355.39): calcd: C, 40.56; H, 3.69; N, 19.71%; found: C, 40.53; H, 3.65; N, 19.69%.

**Synthesis of 4-(3-amino-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-N-(thiazol-2-yl)benzenesulfonamide (5)**

A mixture of compound **2** (0.4 g, 0.0011 mol) and acetic anhydride (25 mL) was heated in water bath for 8 h. The solid material was separated by filtration, crystallization from hot ethanol to give compound **5** . Reddish brown powder; mp 300–302 °C; yield 60%; IR (KBr) ν/cm-1: 3503 (NH), 3230, 3103 ($NH_2$), 1667 (CO), 1373 ($SO_2$); 1H NMR (400 MHz, DMSO-d6): δH ppm 3.25 (s, 2H, CH2, pyrazolone H-4), 6.82 (d, 1H, thiazole H-5, J= 4.4 Hz), 7.25 (d, 1H, thiazole H-4, J= 4.4 Hz), 7.29-7.96 (m, 4H, Ar-H), 12.86 (s, 1H, $NHSO_2$).

MS m/z (%): 337 (M+, 11.02), 327 (12.64), 311 (16.83), 309 (11.41), 294 (9.95), 262 (28.56), 234 (12.71), 226 (13.78), 199 (38.03), 140 (33.13), 138 (21.62), 110 (11.63), 85 (20.39), 73 (82.80), 60 (54.60), 49 (36.60), 44 (100). Anal. for $C_{12}H_{11}N_5O_3S_2$ (337.38): calcd: C, 42.72; H, 3.29; N, 20.76%; found: C, 42.69; H, 3.25; N, 20.73%.

**Synthesis of 2-(4-oxo-4,5-dihydrothiazol-2-yl)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)acetamide (7)**

A mixture of Thioglycolic acid (0.07 mL, 0.0012 mol) in glacial acetic acid (20 mL), compound **1**(0.4 g, 0.0012 mol) was refluxed for 6h. The yield of solid material was obtained in cooling to room temperature was crystallized from hot ethanol to give compound **7**.White powder; mp 295–297 °C; yield 90%; IR (KBr) ν/cm-1: 3500, 3295 (2NH), 1675 (2CO), 1373 (SO2); $^1$H-NMR (400 MHz, DMSO-d6): δH ppm 2.07 (s, 2H, $CH_2$), 3.18 (s, 2H, $CH_2$, thiazolinone H-5), 6.81 (d, 1H, thiazole H-5, J= 4.4 Hz), 7.24 (d, 1H, thiazole H-4, J= 4.4 Hz), 7.7 (m, 4H, Ar-H), 10.26 (s, 1H, NHCO), 12.68 (s, 1H, $NHSO_2$).MS m/z (%): 396 (M+, 1.67), 378 (4.43), 376 (6.70), 362 (25.32), 349 (15.76), 330 (18.94), 328 (46.41), 299 (25.89), 297 (49.48), 287 (12.28), 270 (10.60), 233 (2.73), 232 (28.23), 207 (11.95), 201 (26.44), 190 (16.66), 171 (12.58), 156 (21.56), 142 (29.17), 135 (19.21), 117 (19.74), 109 (24.75), 108 (48.28), 99 (21.84), 93 (19.36), 73 (50.09), 71 (54.27), 65 (61.51), 44 (71.51), 43 (100). Anal. for $C_{14}H_{12}N_4O_4S_3$ (396.46): calcd: C, 42.41; H, 3.05; N, 14.31%; found: C, 42.38; H, 3.02; N, 14.28%.

**Synthesis of 2-(4,6-diamino-3,5-dicyanopyridin-2-yl)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)acetamide (8)**

**Method A.** A mixture of **1** (0.4 g, 0.0012 mol) and malononitrile (0.16 g, 0.0024 mol) was refluxed in boiling ethanol (25 mL) with few drops of piperidine (0.5 mL) for 3 h. The solid material was filtered and crystallized from ethanol to give **8**.

**Method B.** A mixture of **1** (0.4 g, 0.0012 mol) and malononitrile dimer (0.16 g, 0.0012 mol) with few drops of piperidine (0.5 mL) was heated at 140°C in pressure tube in silicon oil bath for 0.5 h. The separated solid material was washed with ethanol and recrystallization from EtOH to afford **8**. Black powder; mp >300°C; yield 90%; IR (KBr) ν/cm-1: 3441 (2NH), 3340, 3225 ($2NH_2$), 2197 (2CN), 1621 (CO), 1315 ($SO_2$). MS m/z (%): 454 (M+, 1.52), 452 (7.18), 451 (4.47), 423 (4.12), 394 (5.84), 368 (30.74), 341 (13.60), 327 (16.68), 313 (59.78), 299 (30.05), 297 (13.66), 285 (10.59), 264 (19.13), 239 (22.18), 236 (147.82), 170 (16.78), 145 (10.20), 123 (19.68), 111 (26.72), 98 (35.48), 97 (51.52), 84 (59.27), 71 (61.96), 69 (92.63), 57 (94.54), 43 (100). Anal. for $C_{18}H_{14}N_8O_3S_2$ (454.49): calcd: C, 47.57; H, 3.11; N, 24.66%; found: C, 47.54; H, 3.08; N, 24.62%.

**Synthesis of 2-imino-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)-2H-chromene-3-carboxamide (9)**

A mixture of compound **1** (0.4 g, 0.0012 mol) and salicylaldehyde (0.13 ml, 0.0012 mol) was refluxed in boiling ethanol (25 mL) with few drops of piperidine (0.5 mL) for 3 h. The obtained solid material on cooling was separated and recrystallized from ethanol to give **9**. Orange crystals; mp 295-297 °C; yield 90%; IR (KBr) ν/cm-1: 3445, 3319, 3252 (3NH), 1675 (CO), 1377(SO2); $^1$H-NMR (400 MHz, DMSO-d6): δH ppm 6.84 (d, 1H, thiazole H-5, J= 4 Hz), 7.26 (d, 1H, thiazole H-4, J= 4 Hz), 7.28-7.82 (m, 7H, Ar-H), 8.58 (s, 1H, chromene CH=), 9.33 (s, 1H, =NH), 12.74 (s, 1H, NHCO), 13.14 (s, 1H, $NHSO_2$). MS m/z (%): 426 (M+0.96 ,), 402 (1.71), 362 (5.91), 342 (0.74), 295 (1.78), 280 (3.36),

255 (8.68), 191 (40.30), 156 (35.65), 143, (22.46), 140 (17.27), 108 (54.74), 92 (100), 77 (26.15), 65 (92.98), 55 (60.18). Anal. for $C_{19}H_{14}N_4O_4S_2$ (426.47): calcd.: C, 53.51; H, 3.31; N, 13.14%; found: C, 53.49; H, 3.29; N, 13.12%.

**Synthesis of (E)-2-cyano-2-(3,3-dimethyl-5-oxocyclohexylidene)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)acetamide (10)**

A mixture of cyanoacetanilide **1** (0.4 g, 0.0012 mol) and dimedone (0.17 mL, 0.0012 mol) was refluxed in boiling EtOH (25 mL) with 4 drops of piperidine for 3 h. The solid material was separated and recrystallization from dry EtOH to afford **10**. Deep yellow powder; mp 270-272 °C; yield 85%; IR (KBr) ν/cm-1: 3445 (2NH), 2188 (CN), 1702, 1616 (2CO). MS m/z (%): 444 (M+3.34 ,), 409 (1.14), 383 (0.92), 348 (7.43), 326 (5.22), 303 (5.09), 282 (6.08), 279 (25.20), 274 (1.85), 247 (4.42), 234 (4.02), 223 (2.91), 189 (4.89), 178 (10.01), 159 (9.43), 138 (12.65), 100 (66.32), 78 (100), 72 (19.78), 62 (17.97). Anal. for $C_{20}H_{20}N_4O_4S_2$ (444.52): calcd.: C, 54.04; H, 4.54; N, 12.60%; found: C, 54.02; H, 4.52; N, 12.50%.

**Synthesis of 2-amino-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (11)**

To a solution of compound **1** (0.4 g, 0.0012 mol) in absolute EtOH (20 mL) including catalytically morpholine (0.5 mL), cyclohexanone (0.0012 mol), and elemental sulfur (0.04 g, 0.0012 mol) were added. Continuously stirring reaction at 60 °C was conducted for 3 h, and then the reaction contents were added drop wise to a crushed ice/water blend acidified by few drops of hydrochloric acid. The yielded precipitate that formed was isolated by filtration, air dried, and recrystallized via a mixture of DMF and ethanol (3:1) to afford compound **11**. Reddish brown powder; mp 290-292 °C; yield 72%; IR (KBr) ν/cm-1: 3443

(2NH), 3235, 3100 ($NH_2$), 1665 (CO); $^1$H-NMR (400 MHz, DMSO-d6): δH ppm 1.19 (m, 2H, $CH_2$), 2.74 (br s, 2H, $CH_2$), 2.90 (br s, 2H, $CH_2$), 3.1 (m, 2H, CH2), 6.82 (d, 1H, thiazole H-5, J= 4 Hz), 7.25 (d, 1H, thiazole H-4, J= 4 Hz), 7.62-7.88 (m, 4H, Ar-H), 7.96 (s, 2H, NH2), 9.22 (s, 1H, NHCO), 12.72 (s, 1H, NHSO2). MS m/z (%): 434 (M+4.76 ,), 421 (5.43), 412 (2.14), 402 (6.53), 378 (5.65), 301 (11.20), 278 (12.67), 245 (11.03), 211 (8.60), 193, (76.97), 165 (12.40), 146 (53.21), 137 (65.81), 119 (34.19), 97 (100), 86 (59.55), 71 (43.32). Anal. for $C_{18}H_{18}N_4O_3S_3$ (434.55): calcd.: C, 49.75; H, 4.18; N, 12.89%; found: C, 49.77; H, 3.6 3.9.

**General procedure for coupling reaction of 1 with different primary aromatic amine diazonium salts**

**a- Preparation of diazonium salt:** A solution of sodium nitrite (0.09 g, 0.0012 mol) in cold water (5 mL) was added drop wise to ice cold solution of the appropriate aromatic amine (0.0012 mol) namely 3-amino-4,6-dimethyl-1H-pyrazolo[3,4-b]pyridine, 2-amino-1H-benzo[d]imidazole, 4-aminoantipyrine and 4-aminoazobenzene containing adequate amount of hydrochloric acid (1.5 mL) was left to stand in ice cold bath for 1 h.

**b- Coupling reaction:** To a cold solution of **1** (0.4 g, 0.0012 mol) in pyridine (20 mL) was added to appropriate diazonium chloride solution drop wise over a period 25 min. with continuous stirring. The reaction mixture was left overnight in refrigerator. The separate solid material was filtered and recrystallized from ethanol to give compounds **12, 14, 16a** and **16b**, respectively.

**Synthesis of (E)-N-(4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-3-yl)-2-oxo-2-((4-(N-(thiazol-2-yl)sulfamoyl)phenyl)amino)acetohydrazonoyl cyanide (12)**

Black powder; mp 295-297 °C; yield 87%; IR (KBr) ν/cm-1: 3479 (4NH), 2216 (CN), 1666 (CO). MS m/z (%): 495 (M+1.43 ,), 459 (0.97), 434 (4.15), 362 (3.81), 303 (4.41), 298 (0.96), 282 (9.59), 259 (25.48), 238 (4.64), 216 (0.98), 212 (6.80), 185 (4.15), 182 (7.11), 170 (9.69), 147 (12.76), 131 (23.41), 104 (12.87), 91 (22.89), 79 (89.31), 52 (64.14), 44 (100). Anal. for $C_{20}H_{17}N_9O_3S_2$ (495.54): calcd.: C, 48.48; H, 3.46; N, 25.44%; found: C, 48.45; H, 3.44; N, 25.41%.

**Synthesis of (E)-2-(2-(1H-benzo[d]imidazol-2-yl)hydrazono)-3-(l2-azanylidene)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)-3l3-propanamide (13)**

Reddish brown powder; mp 290-292 °C; yield 90%; IR (KBr) ν/cm-1: 3446 (4NH), 2214 (CN), 1624 (CO). MS m/z (%): 466 (M+1.18 ,), 440 (0.72), 437 (0.93), 390 (1.39), 368 (4.51), 354 (3.02), 340 (2.51), 265 (4.50), 236 (3.03), 199 (3.93), 181 (1.92), 145 (2.04), 135 (5.96), 125 (3.99), 113 (5.07), 108 (4.30), 107 (9.63), 98 (25.09), 84 (22.01), 83 (31.20), 71 (44.08), 69 (45.59), 43 (100). Anal. for $C_{19}H_{14}N_8O_3S_2$ (466.49): calcd.: C, 48.92; H, 3.03; N, 24.02%; found: C, 48.90; H, 3.01; N, 24.00%.

**Synthesis of 4-imino-8,10-dimethyl-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)-4,6-dihydropyrido[2',3':3,4]pyrazolo[5,1-c][1,2,4]triazine-3-carboxamide (14)**

Heating of **12** (0.53 g, 0.0012 mol) in glacial acetic acid (25 mL) for 3 h, afforded on cooling solid material recrystallized from a mixture of EtOH-DMF (1:1) to furnish compound **14**. Brown powder; mp >300 °C; yield 90%; IR (KBr) ν/cm-1: 3479 (4NH), 1666 (CO); $^1$H-NMR (400 MHz, DMSO-d6): δH ppm 2.67 (s, 3H, CH3-pyridine), 2.74 (s, 3H, CH3-pyridine), 6.84 (d, 1H, thiazole H-5, J= 4 Hz), 7.26 (d, 1H, thiazole H-4, J= 4 Hz), 6.98 (s, 1H, pyridine H-3), 7.43-8.18 (m, 4H, Ar-H), 10.25 (s, 1H, NHCO), 12.23 (s,

1H, =NH) 12.73 (s, 1H, NHSO2). Anal. for $C_{20}H_{17}N_9O_3S_2$ (495.54): calcd.: C, 48.48; H, 3.46; N, 25.44%; found: C, 48.47; H, 3.45; N, 25.43%.

**Synthesis of 4-imino-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)-4,10 dihydrobenzo[4,5]imidazo[2,1-c][1,2,4]triazine-3-carboxamide (15)**

A mixture of **13** (0.53 g, 0.0012 mol) and glacial acetic acid (20 mL) was refluxed for 3 h, and then left to cool. The obtained solid material was separated and recrysrallized from a mixture of EtOH-DMF (1:1) to furnish compound **15**. Orange powder; mp 285-287 °C; yield 88%; IR (KBr) ν/cm-1: 3446 (4NH), 1624 (CO), 1375 (SO2); $^1$H-NMR (400 MHz, DMSO-d6): δH ppm 7.02 (d, 1H, thiazole H-5, J= 4 Hz), 7.28 (d, 1H, thiazole H-4, J= 4 Hz), 7.64-7.9 (m, 8H, Ar-H), 10.14 (s, 1H, NHCO), 12.16 (s, 1H, =NH), 13.19 (s, 1H, $NHSO_2$). Anal. for $C_{19}H_{14}N_8O_3S_2$ (466.49): calcd.: C, 48.92; H, 3.03; N, 24.02%; found: C, 48.91; H, 3.04; N, 24.05%.

**Synthesis of (E)-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-2-oxo-2-((4-(N-(thiazol-2-yl)sulfamoyl)phenyl)amino)acetohydrazonoyl cyanide (16a)**

Deep yellow crystals; mp 260-262 °C; yield 90%; IR (KBr) ν/cm-1: 3526 (3NH), 2212 (CN), 1631, 1591 (2C=O); $^1$H-NMR (400 MHz, DMSO-d6): δH ppm 2.59 (s, 3H, $CH_3$-pyrazolone), 3.18 (s, 3H, $NCH_3$), 6.83 (br s, 1H, thiazole H-5), 7.26 (br s, 1H, thiazole H-4), 7.38-8.03 (m, 9H, Ar-H), 12.73 (s, 1H, $NHSO_2$). MS m/z (%): 536 (M+0.89 ,), 521 (1.14), 391 (0.90), 368 (6.69), 313 (6.41), 236 (9.65), 188 (6.82), 137 (7.45), 129 (10.96), 109 (13.25), 98 (31.53), 83 (44.56), 79 (64.80), 69 (77.35), 57 (100), 43 (39.94). Anal. for $C_{23}H_{20}N_8O_4S_2$ (536.59): calcd.: C, 51.48; H, 3.76; N, 20.88%; found: C, 51.45; H, 3.75; N, 20.86%.

**Synthesis of (E)-2-oxo-N-(4-((E)-phenyldiazenyl)phenyl)-2-((4-(N-(thiazol-2-yl)sulfamoyl)phenyl)amino)acetohydrazonoyl cyanide (16b)**

Reddish brown powder; mp 298-300 °C; yield 95%; IR (KBr) ν/cm-1: 3448, 3381, 3250 (3NH), 2216 (CN), 1686 (C=O); $^1$H-NMR (400 MHz, DMSO-d6): δH ppm 6.84 (br s, 1H, thiazole H-5), 7.26 (br s, 1H, thiazole H-4), 7.56-8.08 (m, 13H, Ar-H), 10.33 (s, 1H, NHCO), 12.26 (s, 1H, =N-NH), 12.70 (s, 1H, $NHSO_2$). MS m/z (%): 530 (M+0.75 ,), 520 (5.15), 462 (2.04), 448 (6.14), 435 (4.94), 423 (2.06), 368 (5.11), 339 (2.73), 324 (5.80), 309 (5.56), 258 (8.80), 236 (5.68), 157 (8.13), 149 (15.19), 135 (15.49), 95 (25.47), 69 (100), 55 (68.32). Anal. for $C_{24}H_{18}N_8O_3S_2$ (530.58): calcd.: C, 54.33; H, 3.42; N, 21.12%; found: C, 54.31; H, 3.39; N, 21.09%.

**General procedure for the synthesis of thiazole derivatives 18-20**

To a solution of compound **1** (0.4 g, 0.0012 mol) in a mixture of KOH (0.07 g, 0.0012 mol) and DMF (25 mL) was stirred for 30 min. phenyl isothiocyanate (0.14 mL, 0.0012 mol) was added. After stirring for 6 h at room temperature α-haloketone derivatives [namely phenacyl bromide (0.25 g, 0.0012 mol) and chloroacetyl chloride (0.1 mL, 0.0012 mol)] were added. The reaction mixture was stirred for 3 h more, then poured into ice cold water. The isolated solid material was recrystallized from EtOH to yield **18** and **20**, respectively.

**Synthesis of (E)-2-cyano-2-(3,4-diphenylthiazol-2(3H)-ylidene)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)acetamide (18)**

Off white powder; mp 180-182 °C; yield 92%; IR (KBr) ν/cm-1: 3459, 3264 (2NH), 2170 (CN), 1699 (CO); $^1$H-NMR (400 MHz, DMSO-d6): δH ppm 5.67 (s, 1H, =CH-thiazole), 6.82 (br s, 1H, thiazole H-5), 7.25 (br s, 1H, thiazole H-4), 7.34-8.04 (m, 14H, Ar-H), 10.22 (s, 1H, NHCO), 11.50 (s, 1H, $NHSO_2$). MS m/z (%): 557 (M+0.99 ,), 534 (1.18), 522 (0.75), 489 (0.92), 409 (0.91), 369 (4.94), 327 (13.72), 313 (6.41), 285 (3.51), 269 (6.33), 236 (10.02), 180 (6.02), 135 (31.36), 111 (18.02), 105 (39.63), 97 (60.19), 77 (66.09), 69 (90.12), 57 (100). Anal. for $C_{27}H_{19}N_5O_3S_3$ (557.07): calcd.: C, 58.15; H, 3.43; N, 12.56%; found: C, 58.12; H, 3.41; N, 12.54%.

**Synthesis of 4-amino-5-benzoyl-2-(phenylamino)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)thiophene-3-carboxamide (19)**

To a solution of compound **1** (0.4 g, 0.0012 mol) in a mixture of KOH (0.07 g, 0.0012 mol) and DMF (25 mL) was stirred for 30 min. phenyl isothiocyanate (0.14 mL, 0.0012 mol) was added. After stirring for 6 h at room temperature phenacyl bromide (0.25 g, 0.0012 mol) was added. The reaction mixture was refluxed for 3 h in presence of TEA (0.5 mL). The reaction mixture was poured into ice cold water. The isolated solid material was recrystallized from EtOH to yield **19**. Deep yellow powder; mp 200-202 °C; yield 89%; IR (KBr) ν/cm-1: 3444 (3NH), 3274, 3150 ($NH_2$), 1660 (2CO); $^1$H-NMR (400 MHz, DMSO-d6): δH ppm 5.67 (s, 2H, $NH_2$), 6.82 (br s, 1H, thiazole H-5), 7.25 (br s, 1H, thiazole H-4), 7.39-7.97 (m, 14H, Ar-H), 11.49 (s, 1H, NHPh), 11.49 (s, 1H, NHCO), 12.67 (s, 1H, $NHSO_2$). MS m/z (%): 575 (M+4.26 ,), 570 (6.36), 530 (19.10), 482 (6.79), 426 (11.98), 399 (11.60), 371 (15.16), 354 (16.16), 331 (30.01), 320 (59.07), 293 (18.80), 282 (23.41), 260 (61.73), 233 (60.23), 210 (23.62), 175 (28.79), 168 (26.32), 146 (18.56), 122 (26.54),

102 (32.56), 80 (83.23), 72 (74.90), 54 (100). Anal. for $C_{27}H_{21}N_5O_4S_3$ (575.68): calcd.: C, 56.33; H, 3.68; N, 12.17%; found: C, 56.31; H, 3.66; N, 12.15%.

**Synthesis of (E)-2-cyano-2-(5-oxo-3-phenylthiazolidin-2-ylidene)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)acetamide (20)**

Reddish brown powder; mp 230-232 °C; yield 96%; IR (KBr) ν/cm-1: 3381, 3340 (2NH), 2194 (CN), 1748, 1728 (2CO); $^1$H-NMR (400 MHz, DMSO-d6): δH ppm 4.03 (s, 2H, $CH_2$-thiazolidinone), 6.82 (d, 1H, thiazole H-5, J=4.4 Hz), 7.25 (d, 1H, thiazole H-4, J=4.4 Hz), 7.43-7.59 (m, 5H, Ar-H), 7.70 (d, 2H, Ar-H, J= 9.2 Hz), 7.74 (d, 2H, Ar-H, J= 8.8 Hz), 9.77 (s, 1H, NHCO), 12.70 (s, 1H, $NHSO_2$). MS m/z (%): 497 (M+2.09 ,), 488 (5.70), 476 (11.44), 440 (6.77), 418 (17.28), 394 (16.17), 369 (14.99), 367 (20.23), 355 (44.86), 337 (48.77), 326 (13.33), 298 (35.46), 268 (100), 264 (34.65), 233 (52.80), 220 (33.22), 219 (30.26), 193 (65.59), 160 (59.56), 147 (29.74), 103 (93.37), 88 (35.20). Anal. for $C_{21}H_{15}N_5O_4S_3$ (497.56): calcd.: C, 50.69; H, 3.04; N, 14.08%; found: C, 50.66; H, 3.01; N, 12.05%.

**Synthesis of (E)-2-cyano-3-(methylthio)-3-(phenylamino)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)acrylamide (21)**

To a solution of **1** (0.4 g, 0.0012 mol) in DMF (25 mL) and KOH (0.07 g, 0.0012 mol), phenyl isothiocyanate (0.14 mL, 0.0012 mol) was added and the reaction mixture was stirred for 6 h. Dimethyl sulfate (0.11 mL, 0.0012 mol) was added and stirring with continued for 3 h more, then poured into ice cold water. The obtained product was filtered and recrystallizrd from EtOH to give compound **21**. Beige powder; mp 200-202 °C; yield 95%; IR (KBr) ν/cm-1: 3433, 3274, 3151 (3NH), 2196 (CN), 1635 (CO), 1368 ($SO_2$); $^1$H-NMR (500 MHz, DMSO-d6): δH ppm 2.27 (s, 3H, $SCH_3$), 6.82 (d, 1H, thiazole H-5, J=

4.4 Hz), 7.24 (d, 1H, thiazole H-4, J= 4.4 Hz), 7.19-7.36 (m, 5H, Ar-H), 7.65 (d, 2H, Ar-H, J= 8.8 Hz), 7.68 (d, 2H, Ar-H, J= 8.8 Hz), 9.89 (s, 1H, NHCO), 11.50 (s, 1H, NHPh), 12.68 (s, 1H, $NHSO_2$); $^{13}C$ NMR (125 MHz, DMSO-d6): δC ppm 16.42, 106.56, 108.08, 118.43, 120.19, 123.50, 124.35, 126.00, 126.67, 129.23, 136.66, 138.65, 141.70, 164.23, 167.49, 168.72.Also DEPT $^{13}C$ NMR, H-H COSY, HSQC and HMBC are in agreement with the proposed structure. MS m/z (%): 471 (M+0.78 ,), 431 (1.03), 400 (1.25), 397 (2.91), 391 (1.15), 365 (1.71), 354 (1.77), 332 (2.61), 281 (2.78), 269 (3.51), 255 (3.78), 191 (18.03), 163 (6.05), 156 (15.66), 143 (9.62), 118 (10.75), 108 (37.93), 92 (70.99), 90 (41.27), 65 (100), 55 (59.27). Anal. for $C_{20}H_{17}N_5O_3S_3$ (471.58): calcd.: C, 50.94; H, 3.63; N, 14.85%; found: C, 50.90; H, 3.59; N, 14.82%.

**Synthesis of 7-amino-5-(phenylamino)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (22)**

An equimolar amount of **21** (0.4 g, 0.0008 mol) and 3-amino-1H-1,2,4-triazole (0.07 g, 0.0008 mol) was dissolved in pyridine (25 mL) and refluxed for 3 h then left to cool. The reaction mixture was cooled in ice cold water and acidified by few drops of HCl. The isolated solid material was recrystallizrd from EtOH to afford **22**.Reddish brown powder; mp 290-292 °C; yield 60%; IR (KBr) ν/cm-1: 3400 (3NH), 3274, 3150 ($NH_2$), 1635 (CO). MS m/z (%): 507 (M+, 1.23), 499 (1.51), 467 (9.99), 434 (3.00), 411 (22.64), 410 (10.90), 371 (4.72), 366 (9.54), 336 (15.37), 332 (21.56), 331 (12.83), 295 (14.55), 269 (53.90), 259 (2.99), 243 (2.45), 193 (4.64), 177 (4.83), 161 (19.31), 156 (49.30), 114 (38.71), 108 (100), 80 (21.05), 59 (42.35), 55 (54.29). Anal. for $C_{21}H_{17}N_9O_3S_2$ (507.55): calcd.: C, 49.70; H, 3.38; N, 24.84%; found: C, 47.67; H, 3.35; N, 24.81%.

**Synthesis of 2-cyano-2-(imidazolidin-2-ylidene)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)acetamide (23)**

Equimolar mixture of compound **21** (0.4 g, 0.0008 mol) and ethylenediamine (0.05 g, 0.0008 mol) in absolute ethanolic (25 mL) solution, was heated for 3 h, then left to cool. The yielded precipitate was isolated by filtration, and recrystallized from EtOH to afford **23**. Beige powder; mp 280-282 °C; yield 75%; IR (KBr) ν/cm-1: 3492, 3399, 3354, 3297 (4NH), 2188 (CN), 1666 (CO); $^1$H-NMR (400 MHz, DMSO-d6): δH ppm 3.45 (s, 4H, 2$CH_2$-imidazolidine), 6.86 (d, 1H, thiazole H-5, J= 4.8 Hz), 7.36 (d, 1H, thiazole H-4, J= 4.8 Hz), 7.66 (d, 2H, Ar-H, J= 9.2 Hz), 7.70 (d, 2H, Ar-H, J= 9.2 Hz), 8.99 (s, 1H, NHCO).MS m/z (%): 390 (M+0.28 ,), 381 (0.89), 367 (0.24), 350 (0.87), 340 (1.80), 339 (16.20), 337 (1.10), 321 (2.40), 313 (15.80), 312 (1.42), 282 (0.71), 279 (1.30), 266 (0.92), 263 (2.53), 255 (1.01), 240 (1.80), 186 (0.21), 179 (0.88), 172 (1.28), 155 (3.02), 144 (78.30), 141 (22.80), 115 (14.30), 97 (12.10), 84 (11.20), 69 (12.30), 57 (42.30), 55 (55.30), 45 (32.80), 44 (71.20), 43 (100). Anal. for $C_{15}H_{14}N_6O_3S_2$ (390.44): calcd.: C, 46.14; H, 3.61; N, 21.53%; found: C, 46.11; H, 3.59; N, 21.49%.

**Synthesis of 5-amino-3-(phenylamino)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)-1H-pyrazole-4-carboxamide (24)**

Hydrazine hydrate (0.04 mL, 0.0008 mol) was added to an ethanolic (25 mL) solution of **21** (0.4 g, 0.0008 mol), the mixture was refluxed for 3 h and then allowed to cool. The solid precipitate that yielded was filtered off and recrystallized from EtOH to afford **24**. White powder; mp 230-232 °C; yield 92%; IR (KBr) ν/cm-1: 3428 (4NH), 3240 ($NH_2$), 1665 (CO); $^1$H-NMR (500 MHz, DMSO-d6): δH ppm 6.07 (s, 2H, $NH_2$), 6.86 (d, 1H, thiazole H-5, J= 5 Hz), 7.36 (d, 1H, thiazole H-4, J= 4.5 Hz), 6.77-7.28 (m, 5H, Ar-H),

7.60 (d, 2H, Ar-H, J= 9 Hz), 7.73 (d, 2H, Ar-H, J= 9 Hz), 8.50 (s, 1H, NHCO), 8.52 (s, 1H, NHPh), 8.98 (s, 1H, NHSO2), 9.04 (s, 1H, NH-pyrazole). MS m/z (%): 455 (M+0.97 ,), 409 (1.06), 368 (3.61), 313 (3.32), 285 (2.85), 256 (4.45), 236 (5.40), 213 (2.93), 185 (3.03), 152 (6.65), 129 (14.09), 109 (15.95), 97 (35.50), 83 (46.56), 71 (46.74), 69 (85.07), 57 (94.89), 55 (100), 41 (67.32). Anal. for $C_{19}H_{17}N_7O_3S_2$ (455.51): calcd.: C, 50.10; H, 3.76; N, 21.53%; found: C, 50.07; H, 3.73; N, 21.49%.

**Synthesis of 5,7-dimethyl-2-(phenylamino)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (25)**

To a (25 ml) glacial acetic acid solution, equimolar mixture of **24** (0.4 g, 0.0009 mol) and acetylacetone (0.092 mL, 0.0009 mol) was added and heated for 3 h, then left to cool, the reaction contents added drop wise to ice cold water. The formed solid material was isolated by filtration, purified by recrystallization from EtOH/benzene to give **25**. Beige powder; mp 220-222 °C; yield 85%; IR (KBr) ν/cm-1: 3439, 3309, 3115 (3NH), 1669 (CO). MS m/z (%): 519 (M+12.03 ,), 508 (5.43), 482 (6.66), 466 (7.66), 459 (6.68), 411 (15.88), 381 (9.97), 362 (5.72), 342 (10.23), 339 (28.12), 334 (20.23), 313 (27.75), 311 (22.06), 296 (13.82), 279 (12.21), 257 (13.52), 239 (26.12), 225 (20.32), 193 (7.58), 156 (11.76), 128 (29.22), 111 (31.74), 85 (49.46), 81 (65.92), 77 (50.19), 57 (63.23), 44 (100). Anal. for $C_{24}H_{21}N_7O_3S_2$ (519.60): calcd.: C, 55.48; H, 4.07; N, 18.87%; found: C, 55.45; H, 4.04; N, 18.84%.

**Coupling of 5-amino pyrazole derivative 24 with malononitrile**

**Synthesis of N-(3-(phenylamino)-4-((4-(N-(thiazol-2-yl)sulfamoyl)phenyl)carbamoyl)-1H-pyrazol-5-yl)carbonohydrazonoyl dicyanide (27)**

**a- Preparation of diazonium salt:**

A solution of sodium nitrite (0.062 g, 0.0009 mol; in 2 ml water) was added drop wise to ice cold solution of compound **24** (0.4 g, 0.0009 mol) in a mixture of acetic acid and conc. HCl [(8:2) 10 ml (1/4) Vol.].

**b- Coupling reaction:**

To a cold solution of malononitrile (0.06 g, 0.0009 mol) in pyridine (10 mL) was added to above formed diazonium chloride solution drop wise over a period 25 min. with continuous stirring. The reaction mixture was left overnight in refrigerator. The separate solid material was filtered and recrystallized from EtOH to give compounds **27**. Deep yellow powder; mp 220-222 °C; yield 65%; IR (KBr) ν/cm-1: 3403, 3313 (5NH), 2198, 2144 (2CN), 1702 (CO). MS m/z (%): 532 (M+2.76 ,), 523 (4.92), 499 (2.85), 441 (17.57), 357 (26.76), 355 (17.85), 320 (11.45), 297 (32.94), 290 (21.82), 281 (7.61), 258 (32.70), 238 (16.71), 191 (20.14), 155 (30.97), 107 (31.41), 92 (64.88), 65 (100), 45 (74.01). Anal. for $C_{22}H_{16}N_{10}O_3S_2$ (532.56): calcd.: C, 49.62; H, 3.03; N, 26.30%; found: C, 49.59; H, 3.00; N, 26.28%.

**Synthesis of 4-amino-3-cyano-7-(phenylamino)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)pyrazolo[5,1-c][1,2,4]triazine-8-carboxamide (28)**

To a (20 ml) glacial acetic acid solution, equimolar mixture of **27** (0.4 g, 0.0008 mol) was added and heated for 3 h, then left to cool, the reaction contents added drop wise to ice cold water. The formed solid material was isolated by filtration, purified by recrystallization from EtOH/DMF to achieve pyrazolo[5,1-c][1,2,4]triazine **28**. Orange powder; mp 240-242 °C; yield 45%; IR (KBr) ν/cm-1: 3403 (3NH), 3313, 3242 ($NH_2$),

2198 (CN), 1702 (CO). MS m/z (%): 532 (M+2.76 ,), 523 (4.92), 499 (2.85), 441 (17.57), 357 (26.76), 355 (17.85), 320 (11.45), 297 (32.94), 290 (21.82), 281 (7.61), 258 (32.70), 238 (16.71), 191 (20.14), 155 (30.97), 107 (31.41), 92 (64.88), 65 (100), 45 (74.01). Anal. for $C_{22}H_{16}N_{10}O_3S_2$ (532.56): calcd.: C, 49.62; H, 3.03; N, 26.30%; found: C, 49.59; H, 3.00; N, 26.28%.

**Synthesis of (E)-2-cyano-3-(4-(dimethylamino)phenyl)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)acrylamide (29)**

An equimolar amount of **1** (0.4 g, 0.0012 mol) and aldehyde 4-N,N-dimethylbenzaldehyde (0.19 g, 0.0012 mol) was dissolved in EtOH (20 mL) including piperidine (0.5 mL) and refluxed for 3 h then left to cool. The obtained solid material was filtered off and recrystallized from EtOH to yield the arylidene **29**. Orange powder; mp 298-300 °C; yield 95%; IR (KBr) ν/cm-1: 3460, 3321 (2NH), 2211 (C≡N), 1677 (C=O); $^1$H-NMR (400 MHz, DMSO-d6): δH ppm 3.02 (s, 6H, N(CH3)2), 6.78 (d, 1H, thiazole H-5, J= 4.8 Hz), 7.22 (d, 1H, thiazole H-4, J= 4.4 Hz), 6.87 (d, 2H, Ar-H, J= 8.8 Hz), 7.78 (d, 2H, Ar-H, J= 9.2 Hz), 7.81 (d, 2H, Ar-H, J= 8.8 Hz), 7.94 (d, 2H, Ar-H, J= 8.8 Hz), 8.09 (s, 1H, vinylic-H), 10.33 (s, 1H, NHCO). MS m/z (%): 453 (M+2.58 ,), 438 (9.45), 409 (3.25), 391 (6.74), 369 (2.63), 347 (10.52), 345 (40.33), 342 (5.61), 325 (18.93), 311 (10.44), 300 (9.90), 270 (12.45), 239 (22.73), 218 (8.55), 199 (22.72), 171 (20.71), 145 (23.04), 111 (33.29), 99 (37.13), 92 (72.60), 73 (48.57), 69 (85.44), 55 (83.78), 45 (100). Anal. for $C_{21}H_{19}N_5O_3S_2$ (453.54): calcd.: C, 55.61; H, 4.22; N, 15.44%; found: C, 55.59; H, 4.19; N, 15.42%.

**Synthesis of 3-amino-5-(4-(dimethylamino)phenyl)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)-1H-pyrazole-4-carboxamide (30)**

Equimolar amounts of of arylidene derivative **29** (0.535 g, 0.0012 mol), and hydrazine hydrate (80%, 0.06mL, 0.0012 mol) in EtOH (20 mL) was refluxed for 3 h, then be cooled to room temperature. The obtained solid material was filtered off and recrystallized from EtOH to afford aminopyrazole **30**. Yellow powder; mp 292-294 °C; yield 62%; IR (KBr) ν/cm-1: 3460 (3NH), 3413, 3321 ($NH_2$), 1677 (C=O). MS m/z (%): 483 (M+0.75 ,), 470 (2.75), 435 (3.24), 410 (1.45), 384 (1.21), 351 (2.33), 327 (1.98), 311 (22.36), 310 (65.82), 309 (100), 294 (21.22), 266 (12.15), 233 (15.45), 205 (23.46), 194 (20.83), 193 (15.18), 164 (29.20), 134 (28.44), 117 (27.65), 91 (28.17), 77 (39.40), 67 (45.16), 65 (35.22), 44 (54.72). Anal. for $C_{21}H_{21}N_7O_3S_2$ (483.57): calcd.: C, 52.16; H, 4.38; N, 20.28%; found: C, 52.15; H, 4.35; N, 20.25%.

**General procedure for the synthesis of pyridin-2-ones 31a-c**

**Method A.** A mixture of **1** (0.4g, 0.0012 mol) and 2-(arylidene)-ethyl cyanoacetate [namely, 2-(4-N,N-dimethylbenzylidene)-ethyl cyanoacetate and/or 2-(4-chlorobenzylidene)-ethyl cyanoacetate and/or 2-(4-methoxybenzylidene)-ethyl cyanoacetate] (0.0012 mol) in dry EtOH (20 mL) with few drops of piperidine (0.5 mL), and the reaction content was refluxed for 3 h, then cooled. The precipitated solid material was filtred and recrystallized from EtOH.

**Method B.** In refluxing ethanol catalyzed with few drops of piperdine (0.5 mL) a mixture of **1** (0.4g, 0.0012 mol) and aromatic aldehydes (namely, 4-N,N-dimethylbenzaldehyde, p-chlorobenzaldehde and p-methoxybenzaldehyde) (0.0012 mol) and ethyl cyanoacetate (0.0012 mol) was heated for 3 h. The reaction content is cooled to room temperature. The isolated precipitate was filtred and recrystallized from EtOH.

**Method C.** Equimolar amounts of **29** (0.001 mol) and ethyl cyanoacetate (0.11 ml, 0.001 mol) in EtOH (20 mL) with few drops piperidine (0.5 mL) was heated for 3 h. The precipitated solid material was filtred and recrystallized from dry EtOH to afford **31a**.

**Synthesis of 4-(3,5-dicyano-4-(4-(dimethylamino)phenyl)-6-hydroxy-2-oxopyridin-1(2H)-yl)-N-(thiazol-2-yl)benzenesulfonamide (31a)**

Orange powder; mp 270-272 °C; yield 92%; IR (KBr) ν/cm-1: 3542 (OH), 3321 (NH), 2212 (2CN), 1677 (CO); 1H NMR (500 MHz, DMSO-d6): δH ppm ppm 3.07 (s, 6H, $N(CH_3)_2$), 6.82 (d, 1H, thiazole H-5, J= 4.5 Hz), 7.25 (d, 1H, thiazole H-4, J= 4.5 Hz), 6.86 (d, 2H, Ar-H, J= 9 Hz), 7.78 (d, 2H, Ar-H, J= 9 Hz), 7.82 (d, 2H, Ar-H, J= 9 Hz), 7.93 (d, 2H, Ar-H, J= 9 Hz), 8.08 (s, 1H, OH), 10.33 (s, 1H, NHSO2).13C NMR (125 MHz, DMSO-d6): δC ppm 39.58, 97.23, 108.12, 111.72, 117.88, 118.51, 119.95, 124.52, 126.76, 133.05, 136.96, 141.95, 151.20, 153.23, 162.19, 168.75. MS m/z (%): 518 (M+4.61 ,), 509 (1.29), 488 (1.68), 475 (3.15), 411 (2.42), 400 (2.26), 370 (4.27), 331 (6.24), 329 (7.20), 298 (5.73), 290 (11.96), 281 (17.91), 257 (33.59), 244 (44.29), 239 (16.87), 216 (29.60), 199 (74.77), 191 (57.94), 171 (74.03), 156 (45.64), 146 (23.38), 118 (41.23), 108 (59.43), 92 (72.50), 65 (82.99), 45 (97.71), 44 (100). Anal. for $C_{24}H_{18}N_6O_4S_2$ (518.57): calcd.: C, 55.59; H, 3.50; N, 16.21%; found: C, 55.58; H, 3.48; N, 16.19%.

**Synthesis of 4-(4-(4-chlorophenyl)-3,5-dicyano-6-hydroxy-2-oxopyridin-1(2H)-yl)-N-(thiazol-2-yl)benzenesulfonamide (31b)**

Yellow powder; mp 290-292 °C; yield 85%; IR (KBr) ν/cm-1: 3543 (OH), 3482 (NH), 2216 (2CN), 1652 (CO). MS m/z (%): 510 (M+ + 1, 2.11), 509 (M+, 5.77), 488 (6.75), 483 (10.43), 456 (19.42), 428 (34.01), 427 (25.99), 404 (9.17), 389 (2.28), 362 (4.10), 345 (4.76), 340 (31.34), 339 (32.41), 323 (66.10), 322 (37.04), 266 (7.28), 262 (14.26), 174

(12.34), 173 (10.75), 121 (100), 48 (29.41). Anal. for $C_{22}H_{12}ClN_5O_4S_2$ (509.94): calcd.: C, 51.82; H, 2.37; N, 13.73%; found: C, 51.80; H, 2.35; N, 13.71%.

**Synthesis of 4-(3,5-dicyano-6-hydroxy-4-(4-methoxyphenyl)-2-oxopyridin-1(2H)-yl)-N-(thiazol-2-yl)benzenesulfonamide (31c)**

Orange powder; mp 295-297 °C; yield 82%; IR (KBr) ν/cm-1: 3507 (OH), 3300 (NH), 2221 (2CN), 1682 (CO). MS m/z (%): 508 (M+ + 3, 20.45), 505 (M+, 61.89), 490 (100), 474 (5.90), 422 (18.67), 405 (8.72), 378 (27.89), 369 (19.68), 350 (21.74), 339 (20.37), 310 (69.18), 282 (9.29), 264 (22.54), 254 (24.47), 245 (43.44), 239 (31.84), 227 (20.79), 204 (25.33), 193 (39.52), 163 (27.47), 156 (47.57), 132 (55.10), 128 (48.11), 109 (28.58), 86 (74.24), 73 (78.43), 69 (65.37). Anal. for $C_{23}H_{15}N_5O_5S_2$ (505.52): calcd.: C, 54.65; H, 2.99; N, 13.85%; found: C, 54.63; H, 2.96; N, 13.82%.

**General procedure for the synthesis of pyridin-2-ones 32a,b**

**Method A.** Equimolar amounts of **1** (0.4g, 0.0012 mol) and the appropriate 2-(arylidene)-malononitrile [namely 2-(4-N,N-dimethylbenzylidene)-malononitrile and 2-(4-chlorobenzylidene)-malononitrile] (0.0012 mol) in EtOH (20 mL) including piperidine (0.5 mL), and the reaction mixture was heated for 3 h. The reaction content was allowed to be cooled. The yielded precipitate that obtained was collected by filtration and recrystallized from ethanol.

**Method B.** Amixture of **1** (0.4g, 0.0012 mol), and the convenient aldehyde (namely 4-N,N-dimethylbenzaldehydeand p-chlorobenzaldehde) (0.0012 mol), piperidine (0.5 mL), and malononitrile (0.002 mol) in hot ethanol (25 mL) for 3 h. The reaction content was

allowed to be cooled. The precipitate that yielded was isolated by filtration, dried and purified by recrystallization from EtOH.

**Method C.** A mixture of **29** (0.001 mol) and malononitrile (0.07 g, 0.001 mol) in EtOH (20 mL) including piperidine (0.5 mL) was heated under reflux for 3 h. The yielded product was isolated by filtration and purified by recrystallization from ethanol to afford **32a**.

**Synthesis of 4-(6-amino-3,5-dicyano-4-(4-(dimethylamino)phenyl)-2-oxopyridin-1(2H)-yl)-N-(thiazol-2-yl)benzenesulfonamide (32a)**

Reddish brown powder; mp 285-287 °C; yield 78%; IR (KBr) ν/cm-1: 3446 (OH), 3411, 3353 ($NH_2$), 2198 (2CN), 1678 (CO); $^1$H-NMR (400 MHz, DMSO-d6): δH ppm ppm 3.08 (s, 6H, $N(CH_3)_2$), 6.49 (d, 1H, thiazole H-5, J= 4 Hz), 6.97 (d, 1H, thiazole H-4, J= 4 Hz), 6.87 (d, 2H, Ar-H, J= 9.2 Hz), 7.68 (d, 2H, Ar-H, J= 8.8 Hz), 7.72 (d, 2H, Ar-H, J= 8.8 Hz), 7.94 (d, 2H, Ar-H, J= 8.8 Hz), 8.08 (s, 2H, $NH_2$), 10.33 (s, 1H, $NHSO_2$). MS m/z (%): 517 (M+, 6.14), 503 (1.58), 491 (5.59), 490 (19.76), 488 (4.35), 487 (2.68), 460 (2.94), 421 (5.30), 395 (2.01), 370 (1.79), 349 (6.35), 332 (16.50), 320 (8.34), 317 (6.55), 290 (4.54), 276 (12.93), 246 (4.13), 223 (4.57), 214 (6.72), 192 (13.47), 156 (12.53), 128 (15.03), 114 (17.44), 75 (100), 68 (37.32), 60 (18.61). Anal. for $C_{24}H_{19}N_7O_3S_2$ (517.58): calcd.: C, 55.69; H, 3.70; N, 18.94%; found: C, 55.67; H, 3.68; N, 18.92%.

**Synthesis of 4-(6-amino-4-(4-chlorophenyl)-3,5-dicyano-2-oxopyridin-1(2H)-yl)-N-(thiazol-2-yl)benzenesulfonamide (32b)**

Pale yellow powder; mp 290-292 °C; yield 83%; IR (KBr) ν/cm-1: 3637 (OH), 3450, 3143 ($NH_2$), 2225, 2213 (2CN), 1674 (CO). MS m/z (%): 508 (M+, 5.69), 503 (9.56), 458 (16.82), 426 (7.52), 393 (10.11), 385 (33.66), 355 (19.97), 330 (24.61), 329 (21.00), 297

(20.94), 269 (7.33), 265 (11.60), 255 (22.33), 240 (29.14), 216 (26.35), 184 (23.29), 156 (30.80), 142 (21.59), 118 (24.75), 113 (53.79), 93 (45.52), 84 (85.19), 77 (95.88), 65 (100), 51 (87.48), 45 (42.06), 41 (51.97). Anal. for $C_{22}H_{13}ClN_6O_3S_2$ (508.96): calcd.: C, 51.92; H, 2.57; N, 16.51%; found: C, 51.89; H, 2.55; N, 16.48%.

**Laboratory Bioassay**

**Cotton leaf worm (Spodoptera littoralis, Family; Lepidoptera)**

Laboratory experiments were conducted to study the insecticidal activity of the newly synthesized tested compounds against the 2$^{nd}$ instar larvae of S. littoralis (Boisd.). A research facility strain of the cotton leafworm S. littoralis (Boisd.) was kept up under steady states of 25 ± 1°C and 70 ± 5% RH and kept off any pollutions by chemicals till the time of treatment to concentrate keeping in mind the end goal to get a susceptible and homogenous strain as depicted by El-Defrawi et al. (1964)[25].

**Toxicological studies:**

It was carried out according to the previously reported method[19, 26-29].

**Biochemical aspects:** Some enzymes activities were estimated in this experimental part of study in the 4$^{th}$ instar larvae of a laboratory *strain of S. littoralis* (Boisd.) after exposure with the tested synthetic compounds. At the $LC_{50}$ value of an aqueous solution of each insecticide, castor bean leaves were dipped in for 30 s, then left to dry in shade at room temperature for 30 min before being presented to the 4th instar larvae of a laboratory strain. For 48 h the larvae were fed on the treated leaves, and then transferred to feed on freshly untreated leaves for three days. From approximately fifty larvae, the haemolymph was acquired by removing one of the prolegs with forceps; gentle pressure was applied on

the larvae with the fingers and extracting the haemolymph with a syringe. The haemolymph was collected in test tubes and stored in a refrigerator until determination of the enzymatic activities[30].

**Determination of enzyme activities**

Alkaline phosphatase (ALK-P) activity was measured according to the described method by Powell & Smith (1954)[31]. The activity of acetylcholine esterase (AchE) was determined according to the described method by Simpson et al. (1964)[32]. The activities of serum esterases including alanine aminotranferase (ALT) and asparate aminotransferase (AST) enzymes were estimated calorimetrically (Reitman and Frankel 1957)[33]. Total proteins were estimated by Bradford's (1976) method[34].

**Histological assay:**

This procedure was followed the reported method[35] to determine the histological effect of compounds **16a, 8, 28** and **31b** on the larval body wall (cuticle), midgut, and fat body and malpighian tubules.

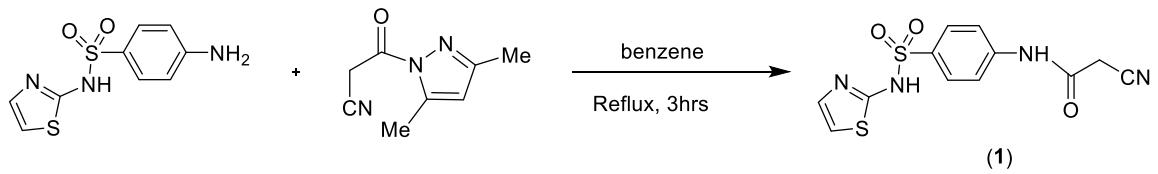
**Biological studies:** Caster bean leaves were soaked in $LC_{25}$ of each tested compound, and used for feeding the newly 4th larval instar. Three hundred larvae were used for each tested compound[35] Then the adult longevity was determined (from adult emergence until adult death for male and female), fecundity (no. of eggs/female), fertility (percentage of eggs hatchability) and fecundity percentage was calculated according to Crystal and Lachance (1963)[36] as follows:

% Fecundity =No. eggs (treated female)/No. eggs (untreated female) x 100

**Statistical analysis:** All biological aspects were analyzed using one-way ANOVA by SPSS 13.0 (SPSS, 2004). Duncan's Multiple Range Test (DMRT) was used to determine the probability level to compare the differences among some parameter means ($P<0.05$) by Costat system for Windows, Version 6.311, Berkeley, CA, USA, Costat program (2006) [37].

## RESULTS AND AISCUSSION

**Chemistry.** The synthetic pathways adopted to obtain the target compounds are outlined in Schemes **1-5**. The known key intermediate, 2-cyano-*N*-(4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl)acetamide **(1)**, was synthesized in high yield by cyanoacetylation of sulfathiazole in dry benzene with 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile according to the previously reported procedure[19] (Scheme **1**).

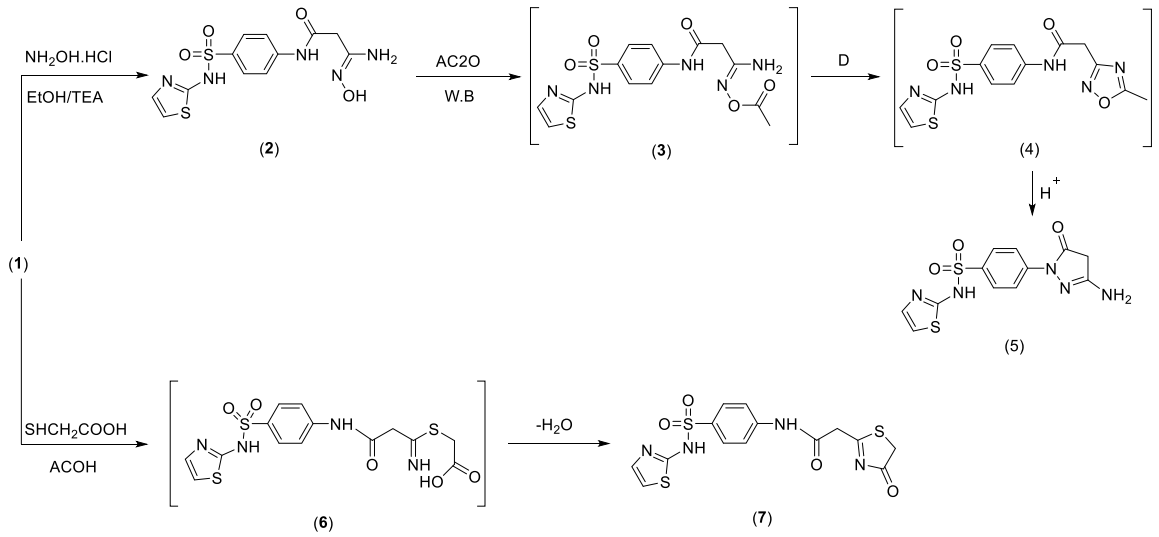



**Scheme 1 Synthesis of starting cyanoacetanilide 1**

The IR spectrum of compound **1** exhibited stretching frequencies at 3459, 3366 $cm^{-1}$ for the 2NH functions, a sharp stretching band at 2258 $cm^{-1}$ for the cyano group, a strong sharp band at 1713 $cm^{-1}$ for the amidic carbonyl group and absorption band at 1339 $cm^{-1}$ characteristic to $SO_2$ group. The MS achieved a parent ion peak at *m/z* 322 ($M^+$), assigned to the molecular formula $C_{12}H_{10}N_4O_3S_2$.

Thus, treatment of the precursor **1** with hydroxylamine containing a catalytically amount of TEA as a base afforded amidoxime **2**. Acetylation of amidoxime **2** with acetic anhydride delivered *O*-acetylation product **3**. Thermal cyclization of **3** furnished 1,2,4-

oxadiazole derivative **4** which underwent acid catalyzation rearrangement to afford pyrazolin-5-one derivative **5** (Scheme 2). The amidoxime **2** was characterized by the absence of nitrile function in its IR spectrum and the appearance of hydroxyl group at absorption band 3512 $cm^{-1}$ and amino function at absorption bands 3341, 3300 $cm^{-1}$ (cf. experimental part).

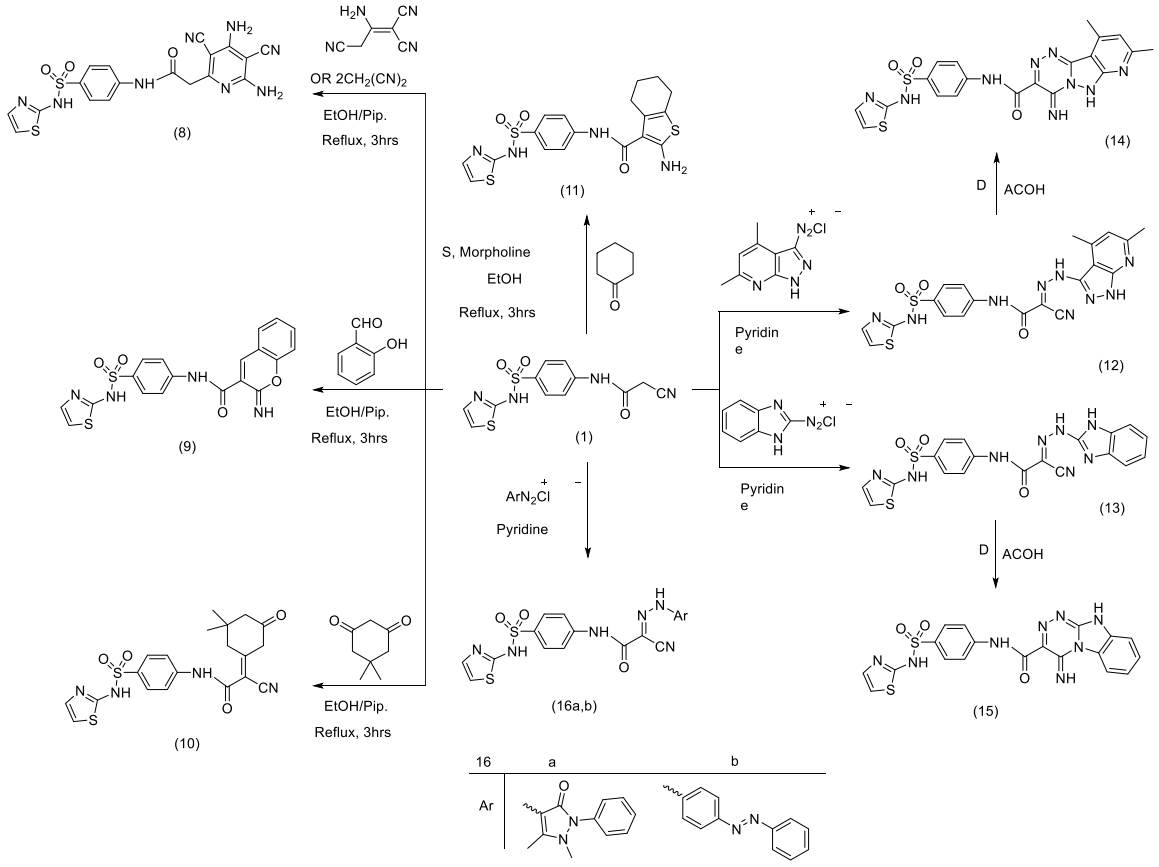



**Scheme 2 Synthesis of pyrazolin-5-one and thiazolinone derivatives**

Cyclocondensation of cyanoacetanilide **1** with 2-mercaptoacetic acid in boiling glacial acetic acid afforded the thiazolinone derivative **7** in high yield. IR spectrum of thiazolinone derivative **7** indicated stretching frequencies at 3500, 3295 $cm^{-1}$ attributed to 2NH groups, while strong absorption band at 1675 $cm^{-1}$ ascribed to 2CO functions. Its $^{1}$H-NMR spectrum (DMSO-$d_6$) indicated .a singlet signal at $\delta_H$ 2.07 ppm assigned to methylene protons and a singlet signal at $\delta_H$ 3.18 ppm equivalent to two protons ascribed to methylene protons of the thiazolinone moiety

We report here the reaction of cyanoacetanilide **1** with malononitrile dimer in refluxing EtOH with a few drops of piperidine to furnish pyridinyl acetanilide **8**. Structure **8** was in

agreement with its spectral data, IR and MS. Knoevenagel buildup of starting compound **1** with salicylaldehyde in hot ethanol including catalytically drops of piperidine accomplished the objective 2-iminochromene derivative **9** (Scheme 3). On the other hand, (*E*)-2-cyano-2-(3,3-dimethyl-5-oxocyclohexylidene)-*N*-(4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl)acetamide **(10)** was synthesized upon interaction of **1** with dimedone in the presence of hot ethanolic piperidine solution (Scheme 3).




**Scheme 3 Synthesis of pyridine, chromene, thiophene, pyrido[2',3':3,4]pyrazolo[5,1-c][1,2,4]triazine, benzo[4,5]imidazo[2,1-c][1,2,4]triazine and arylazo compounds derivatives**

Furthermore, under Gewald reaction conditions, heterocyclization of cyanoacetanilide **1** with both cyclohexanone and elemental sulfur in ethanol upon heating under reflux and in presence catalytically morpholine drops furnished the desired thiophene derivative **11**

(Scheme 3). Elemental analysis, IR and $^1$H-NMR, are in agreement with the proposed structure (cf. experimental part.

The present work describes the preparation of the bridged-head nitrogen heterocyclic systems from a perfect building block, diazotized heterocyclic amines[19]. Consequently, coupling of the key intermediate **1** with both 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-diazonium chloride and 1*H*-benzo[*d*]imidazol-2-diazonium chloride in pyridine at 0-5°C afforded the corresponding hydrazono compounds **12**, **13**,respectively. On heating compounds **12** and **13** in refluxing acetic acid, it cyclized to 4-imino-8,10-dimethyl-*N*-(4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl)-4,6-dihydropyrido[2',3':3,4]pyrazolo[5,1-*c*][1,2,4]triazine-3-carboxamide (**14**) and 4-imino-*N*-(4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl)-4,10-dihydrobenzo[4,5]imidazo[2,1-*c*][1,2,4]triazine-3-carboxamide (**15**), respectively, (Scheme 3).

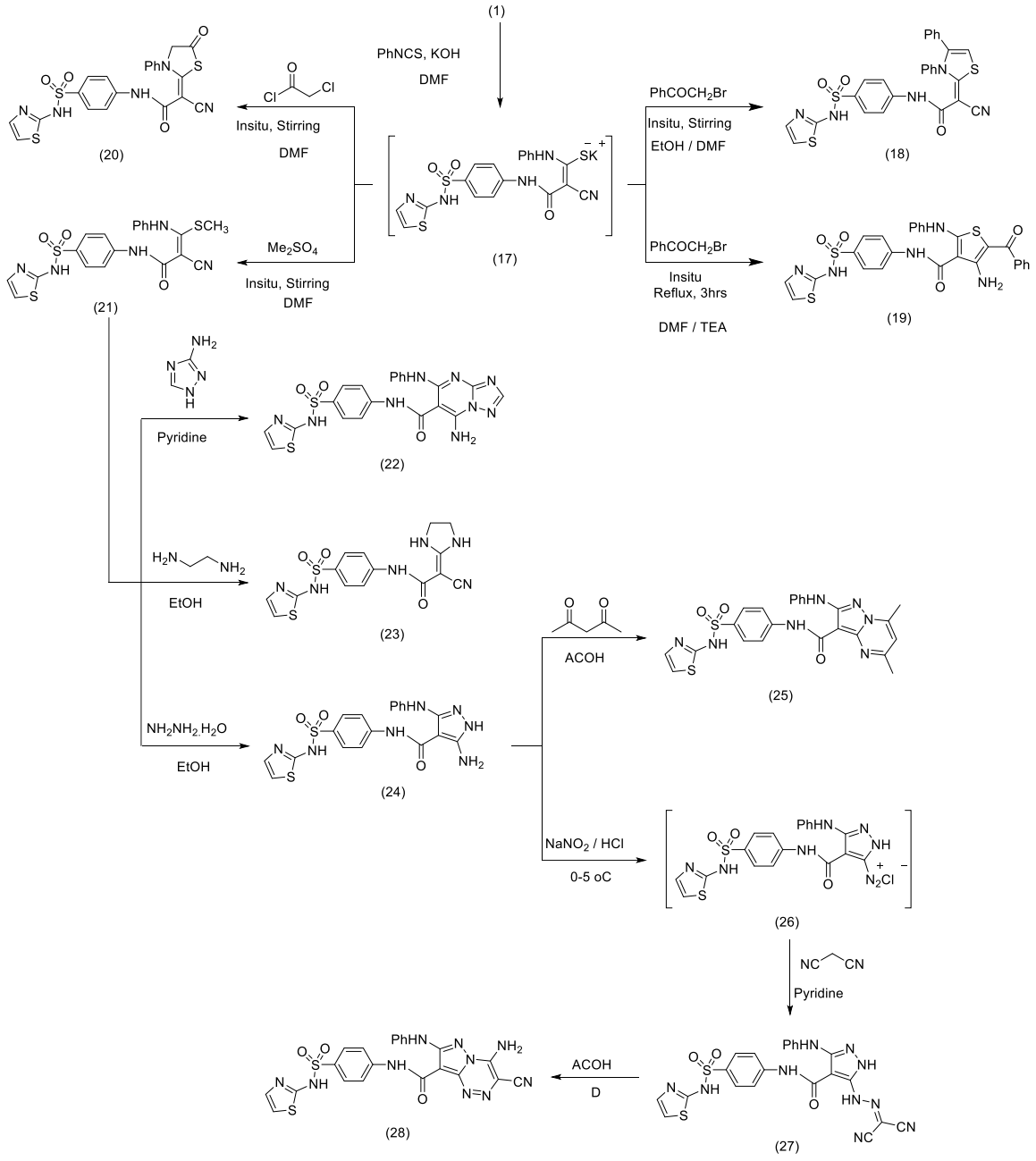
The $^1$H-NMR spectrum (DMSO-$d_6$) of 14 displayed three $D_2O$-exchangeable singlets at $\delta_H$ 10.25, 12.23 and 12.73 ppm due to *N*HCO, =NH and *N*H$SO_2$ protons, additionally, three singlets at $\delta_H$ 2.67, 2.74 and 6.98 ppm characterized for two $CH_3$ protons of pyridine ring, and one aromatic proton of the pyridine ring H-3, respectively. IR spectrum of **15** indicated the lack of cyano group absorption band. Four NH appeared as a broad absorption band at 3446 cm$^{-1}$, while a strong absorption appeared at 1624 cm$^{-1}$ for amidic carbonyl group. The $^1$H NMR spectrum (DMSO-$d_6$) indicated the absence of a singlet signal assignable to methylene protons, Moreover, compound **1** diazocoupled with antipyrine diazonium chloride and azobenzene diazonium chloride in pyridine at 0-5°C to furnish the anticipated highly biologically active hydrazone derivatives **16a** and **16b**, respectively (Scheme 3). Sulfonamide thiazole bearing antipyrine nucleus, **16a** was established by the

appearance of two singlet signals equivalent to six protons at $\delta_H$ 2.59, 3.18 ppm in the $^1$H NMR spectrum (DMSO-$d_6$), which represent the $CH_3$, $NCH_3$ protons of the pyrazolone moiety of antipyrine. IR spectrum of **16a** exhibited stretching frequencies at 3526, 2212, 1631 and 1591 $cm^{-1}$ assignable for (3NH), nitrile function, and two amidic carbonyls.

When **1** was applied to react with phenyl isothiocyanate in DMF in the presence of potassium hydroxide at room temperature furnished the non-isolable intermediate thiocarbamoyl salt **17**.Hence, the *insitu* stirring reaction of the non-isolable intermediate **17** with phenacyl bromide in presence of ethanol/ DMF afforded the thiazole derivatives **18**, (Scheme 4 .On the other hand, when the intermediate enaminonitrile **17** was refluxed with phenacyl bromide in DMF only as aprotic solvent and in the presence of catalytic amount of basic triethylamine, a thiophene derivative **19** was acquired (Scheme 4). The proposed structure of **19** was affirmed by the lack of nitrile function absorption band in the IR spectrum. Reaction of **17** with choloracetyl choloride in DMF afforded thiazolon **20** .The structure of **20** was confirmed by the existence of a singlet signal equivalent to two protons at $\delta_H$ 4.03 ppm in the $^1$H NMR spectrum, which represent the methylene protons at C4 of the thiazolidinone moiety. IR spectrum indicated a new absorption band at 1748 $cm^{-1}$ due to a carbonyl group at C5 of thiazole ring.

When the intermediate thiocarbamoyl salt **17** underwent *insitu* alkylation with $(CH_3)_2SO_4$, the corresponding acrylamide **21** was generated[12] (Scheme 4).. The $^1$H NMR spectrum of 21 (DMSO-$d_6$) displayed a singlet signal at $\delta_H$ 2.27 ppm assignable to $SCH_3$ protons. The $^{13}$C NMR spectrum (DMSO-$d_6$) was identified by signals at $\delta_C$ 16.42 ppm characterized to $SCH_3$ carbon, and a signal at $\delta_C$ 118.43 ppm ascribed to the nitrile carbon. The structure of the ketene *N,S*-acetal **21** was also confirmed by DEPT $^{13}$C NMR and 2D

NMR such as H-H COSY, HSQC and HMBC. The reactivity of acrylamide **21** towards nitrogen nucleophiles was investigated. Subsequently, triazolo[1,5-*a*]pyrimidine derivative **22** was acquired *via* heating of compound **21** with 3-amino-1*H*-1,2,4-triazole in pyridine, (Scheme 4).The IR spectrum indicated the absence of conjugated cyano function absorption band and exhibited absorption bands at 3400, 3274, 3150 and 1635 $cm^{-1}$ assignable to 3NH, $NH_2$, and amidic CO functions, respectively.




**Scheme 4 Reaction of cyanoacetanilide 1 with phenyl isothiocyanate**

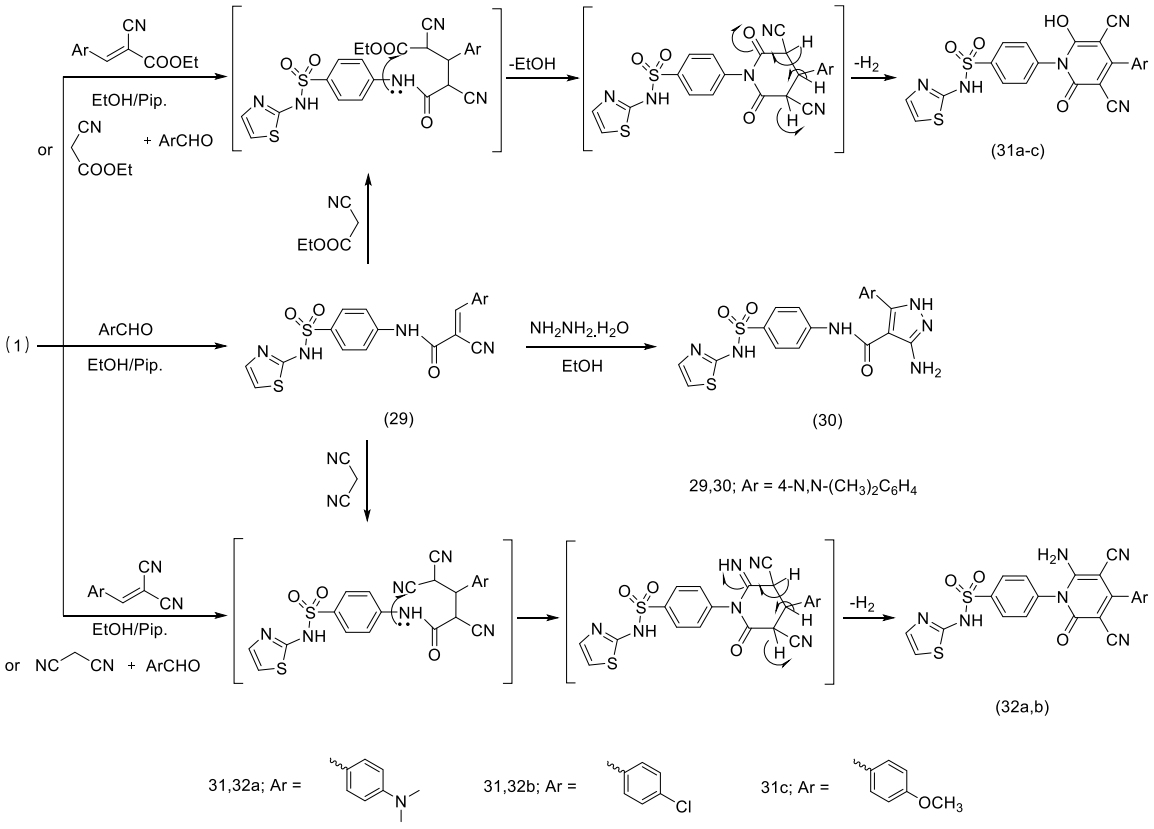
Treatment of **21** with bifunctional nucleophilic reagents such as ethylene diamine in boiling ethanol, afforded the imidazolidine derivative **23** (Scheme 4). Its $^{1}$H-NMR spectrum (DMSO-$d_6$) showed a signal at $\delta_H$ 3.45 ppm equivalent to four protons ascribed to the two methylene protons. Cyclocondensation of the acrylamide **21** with hydrazine

hydrate in EtOH upon heating under reflux achieved the desired 5-aminopyrazole derivatives **24**. $^1$H NMR (DMSO-$d_6$) indicated a singlet signal equivalent for two protons at $\delta_H$ 6.07 ppm attributed to $NH_2$ protons.

Cyclocondensation reaction of aminopyrazole **24** with acetylacetone in glacial acetic acid upon heating under reflux afforded pyrazolo[1,5-*a*]pyrimidine derivative **25** (Scheme 4). Moreover, diazotization of compound **24** with sodium nitrite and conc. HCl furnished the corresponding diazonium chloride **26**, which was followed by coupling with malononitrile in pyridine to yield the desired hydrazono derivatives **27**. When compound **27** was heated in glacial acetic acid, the objective pyrazolo[5,1-c][1,2,4]triazine derivatives **28** was achieved (Scheme 4). The IR spectrum of **27** indicated the azo function at 1573 $cm^{-1}$, broad absorption bands at 3403, 3313 $cm^{-1}$ due to (5NH), two cyano functions at 2198, 2144 $cm^{-1}$ and a carbonyl function at 1702 cm-1

The Knoevenagel condensation of the cyanoacetanilide **1** with 4-*N*,*N*-dimethylbenzaldehyde in refluxing ethanol with few drops of piperidine as a basic catalyst furnished the corresponding arylidene derivative **29** (Scheme 5). Michael addition of hydrazine hydrate to α,β-unsaturated nitrile **29** in boiling ethanol yielded 3-amino-5-(4-(dimethylamino)phenyl)-*N*-(4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl)-1*H*-pyrazole-4-carboxamide **(30)**(Scheme 5). The assignment of structures **29**, **30** was supported by spectral data. The $^1$H NMR (DMSO-$d_6$) spectrum of the acrylamide structure **29** provided three singlet signals at $\delta_H$ 3.02, 8.09 and 10.33 ppm attributable for $N(CH_3)_2$, vinylic and *N*HCO protons, respectively. Furthermore, IR spectrum of aminopyrazole **30** revealed the lack of cyano function and instead, the appearance of a new absorption band at 3413, 3321 $cm^{-1}$ assigned to an $NH_2$ group. One-pot reactions of the cyanoacetanilide derivative **1** with

ethyl cyanoacetate and different aromatic aldehydes namely 4-*N*,*N*-dimethylbenzaldehyde, *p*-chlorobenzaldehyde, and *p*-methoxybenzaldehyde (1:1:1 molar ratio) in refluxing ethanol containing catalytically amount of piperidine yielded the pyridin-2-one derivatives **31a-c**, respectively (Scheme 5). Furthermore, the pyridin-2-one derivatives **31a-c**, were also obtained *via* the reaction of cyanoacetanilide **1** with arylidene ethyl cyanoacetate, in hot ethanol under reflux containing piperidine as a catalyst. The $^{1}$H NMR spectrum of 31a (DMSO-$d_6$) indicated the presence of a singlet signal at $\delta_H$ 3.07 ppm equivalent for six protons due to $N(CH_3)_2$ protons. Its $^{13}$C NMR spectrum (DMSO-$d_6$) revealed the presence of two methyl carbons at $\delta_C$ 39.58 ppm, two cyano carbons at $\delta_C$ 117.88 and 118.51 ppm, thiazole carbons, C5 and C4 at $\delta_C$ 108.12, 124.52, respectively, eight aromatic carbons at $\delta_C$ 126.76-133.05 ppm, in addition to a carbonyl carbon appeared at $\delta_C$ 162.19 ppm. The structure of the 2-pyridone derivative **31a** was also characterized by DEPT $^{13}$C NMR and 2D NMR such as H-H COSY, HSQC and HMBC.




699

700 **Scheme 5 Synthesis of arylidene, aminopyrazole and 2-pyridone derivatives**

701 Another pathway for the synthesis of 2-pyridone derivatives, was the reaction of the
702 cyanoacetanilide derivative **1***via* one-pot reaction, with malononitrile and the aromatic
703 aldehydes, 4-*N*,*N*-dimethylbenzaldehyde, *p*-chlorobenzaldehyde (1:1:1 molar ratio) in
704 boiling ethanol containing few drops of piperidine to furnish pyridinones **32a,b** (Scheme
705 5). Moreover, when arylidene malononitrile refluxed with the cyanoacetanilide derivative
706 **1** in ethanol in the presence of piperidine afforded 2-pyridone derivatives **32a,b**. The $^{1}$H
707 NMR spectrum of **32a** revealed a singlet signal at $\delta_H$ 3.08 ppm for $N(CH_3)_2$ protons. As
708 well, pyridin-2-ones **31a** and **32a** were also obtained *via* the reaction of the arylidene
709 derivative **29** as Michael acceptors with ethyl cyanoacetate and\or, malononitrile,

710 respectively, in boiling ethanol in presence of few drops of piperidine as a basic catalyst
711 (Scheme 5). The structures, **31a-c**, **32a,b** were confirmed on the basis of its spectral data.

712 ***Insecticidal activity***

713 ***Toxicity test for the Cotton leafworm, Spodoptera littoralis.***

714 It was carried out according to reported method [19]. Regarding the determined $LC_{50}$ and
715 $LC_{90}$ values, sulfonamides bearing thiazole moiety **16a**, **8**, **28**, **31b** and **7** showed the most
716 potent toxic effects with $LC_{50}$ values of 49.04, 62.66, 78.62, 94.90 and 105.10 ppm,
717 respectively, and toxicity index being 100, 78.26, 62.38, 51.68 and 46.66%, respectively.
718 It is interesting to note that the insecticidal activities of the tested compounds against the
719 2nd instar larvae of *S. littoralis* (Boisd.) after 3 days of treatment obey the following smooth
720 order:

721 **16a**>**8**>**28**>**31b**>**7**>**47**>**16b**>**31a**>**32b**>**13**>**9**>**15**>**20**>**32a**>**18**>**22**>**1**>**19**>**24**>**25**>**31c**>**23**
722 >**29**>**30**.

723 ***Structure–activity relationship***

724 The structure–activity relationship referred that sulfonamides bearing thiazole moiety
725 derivatives **16a-30** exhibited the highest toxic biological activity [21], this results may be due
726 to the presence of sulfonamides and cyano groups in their structures. Moreover, the
727 presence of electron attracting groups/atoms is significant to increase the insecticidal
728 activity[22] (compounds **13** , **15** and **16a**)

729 **Table 1 Susceptibility of the 2nd instar larvae of the laboratory strain of cotton leafworm, *S. littoralis* (Boisd.) to**
730 **the the newly synthesized compounds as insecticidal agents after 3 days of treatment**

| Tested compounds | $LC_{50}$ (ppm) and confidence limits at 95% | $LC_{90}$ (ppm) and confidence limits at 95% | Slope | Toxicity index % at $LC_{50}$ value |
|---|---|---|---|---|
| **16a** | 49.04<br>22.07 73.73 | 339.13<br>195.12 1397.98 | 1.526+/- 0.293 | 100 |

| Tested compounds | $LC_{50}$ (ppm) and confidence limits at 95% | $LC_{90}$ (ppm) and confidence limits at 95% | Slope | Toxicity index % at $LC_{50}$ value |
|---|---|---|---|---|
| **8** | 62.66 (36.89 – 87.67) | 340.83 (219.53 – 823.12) | 1.742+/- 0.269 | 78.26 |
| **28** | 78.62 (35.56 – 130.78) | 1007.48 (428.88 – 12126.07) | 1.157+/- 0.235 | 62.38 |
| **31b** | 94.90 (47.10 – 161.26) | 1238.71 (499.19 – 18452.28) | 1.149+/- 0.234 | 51.68 |
| **7** | 105.10 (61.79 – 158.12) | 922.36 (486.01 – 3647.67) | 1.359+/- 0.214 | 46.66 |
| **16b** | 124.75 (76.47 – 189.05) | 1098.45 (560.21 – 4777.51) | 1.357+/- 0.215 | 39.31 |
| **5** | 147.95 (94.22 – 227.02) | 1276.07 (634.78 – 5956.18) | 1.370+/- 0.218 | 33.15 |
| **31a** | 174.93 (115.05 – 272.81) | 1441.09 (706.10 – 7005.54) | 1.399+/- 0.222 | 28.03 |
| **32b** | 205.59 (138.83 – 326.11) | 1574.99 (769.20 – 7703.38) | 1.449+/- 0.228 | 23.85 |
| **13** | 206.69 (128.73 – 306.08) | 1609.92 (811.91 – 9304.43) | 1.438+/- 0.261 | 23.73 |
| **9** | 240.88 (157.35 – 364.50) | 1835.10 (899.73 – 11646.17) | 1.453+/- 0.265 | 20.36 |
| **15** | 279.66 (189.59 – 435.94) | 2041.33 (981.98 – 13745.32) | 1.485+/- 0.270 | 17.54 |
| **20** | 283.82 (207.31 – 382.69) | 1426.27 (873.41 – 3846.50) | 1.828+/- 0.264 | 17.28 |
| **32a** | 318.11 (235.53 – 434.59) | 1589.91 (951.85 – 4601.30) | 1.834+/- 0.270 | 15.42 |
| **18** | 355.53 (267.90 – 488.99) | 1675.58 (1002.17 – 4905.63) | 1.904+/- 0.281 | 13.79 |
| **22** | 399.54 (307.35 – 549.79) | 1692.39 1029.04 4810.66 | 2.044+/- 0.302 | 12.27 |
| **1** | 443.66 (342.08 – 628.23) | 1858.24 1102.80 5772.58 | 2.060+/- 0.315 | 11.05 |
| **19** | 488.17 (381.43 – 707.71) | 1815.74 1069.53 7115.06 | 2.247+/- 0.403 | 10.05 |
| **24** | 520.45 (414.66 – 743.70) | 1700.51 (1046.46 – 5740.47) | 2.492+/- 0.436 | 9.42 |
| **25** | 530.83 (430.8 – 733.83) | 1540.91 (997.02 – 4434.11) | 2.769+/- 0.471 | 9.24 |
| **31c** | 553.32 (466.20 – 689.42) | 1386.92 (999.10 – 2764.85) | 3.211+/- 0.476 | 8.86 |
| **23** | 573.23 (459.43 – 844.34) | 1735.59 (1071.98 – 5990.46) | 2.664+/- 0.475 | 8.56 |
| **29** | 580.72 (457.02 – 901.08) | 1944.22 (1137.60 – 8158.85) | 2.442+/- 0.449 | 8.44 |
| **30** | 586.34 (495.86 – 738.45) | 1426.30 (1023.67 – 2918.36) | 3.320+/- 0.504 | 8.36 |

Note: Toxicity index is defined as the ratio of the most effective compound's $LC_{50}$ value to the other tested compound's $LC_{50}$ value multiplying by 100.

***Biochemical impacts***

***Determination of Alkaline phosphatase (Alk-P) activities.*** Data in Table 2 indicated that (**16a**) produced a significantly highest reduction in the activity of alkaline phosphatase (Alk-P) lower than in the control, it was -57.03%, followed by (**8**) and (**28**), of which, it was by -38.74, -29.15% lower than in the control, respectively, while the lowest decrease in Alk-P activity was induced by (**31b**), by -19.70% lower than in the control.

**Table 2 Alkaline phosphatase activity in haemolymph of the 4th instar larvae *S. littoralis* (Boisd.) after 5 days of treatment with $LC_{50}$ of 16a, 8, 28 and 31b**

| Tested compounds | Alkaline phosphatase (U/L) | % of control |
|---|---|---|
| **16a** | $52.73^{e}$±4.96 | -57.03 |
| **8** | $75.17^{d}$±3.13 | -38.74 |
| **28** | $86.93^{c}$±3.38 | -29.15 |
| **31b** | $98.53^{b}$±1.42 | -19.70 |
| **Control** | $122.7^{a}$±1.99 | |
| **LSD=0.05** | 10.151 | |

% of control = (Test – Control)/Control × 100; Letters mean the significant differences between treatments according to Duncan's test
Data are the means ±SE (Standard error) of three replicates of 50 4th larvae each

***Determination of alanine aminotranferase (ALT) and asparate aminotransferase (AST) activities.*** Our results revealed that all the tested compounds showed a significant increase in alanine aminotransferase (ALT)activity (Table 3), the enzyme activity reached its maximum value in **16a** treated larvae (321.13% higher than in the control), and while the enzyme activity was noticed to be at the lowest increase in **31b** treated larvae (100.92% higher than in the control). Compounds **8** and **28** have a remarkable increase in the enzyme

activity by 164.08% and 174.15%, respectively, higher than the control but show no significance between each other. Also, we noticed that there was an elevation in asparate aminotransferase (AST) activity (Table 3). Of the tested compounds **16a** was the most potent insecticidal properties, which showed a highly significant enzyme activity increase (342.11% higher than in the control), followed by **8**(138.74%), **28** (135.18%) then **31b** (64.07%), respectively.

**Table 3 Changes of alanine aminotranferase (ALT) and asparate aminotransferase (AST) activities in haemolymph of the 4th instar larvae *S. littoralis* (Boisd.) after 5 days of treatment with $LC_{50}$ of 16a, 8, 28 and 31b**

| Tested compounds | ALT activity (U/L) | % of control | AST activity (U/L) | % of control |
|---|---|---|---|---|
| **16a** | $59.8^{a}$±3.09 | 321.13 | $70.87^{a}$±1.20 | 342.11 |
| **8** | $37.5^{b}$±1.48 | 164.08 | $38.27^{b}$±0.92 | 138.74 |
| **28** | $38.93^{b}$±2.11 | 174.15 | $37.7^{b}$±0.87 | 135.18 |
| **31b** | $28.53^{c}$±0.38 | 100.92 | $26.3^{c}$±0.46 | 64.07 |
| **Control** | $14.2^{d}$±1.33 | | $16.03^{d}$±2.47 | |
| **LSD=0.05** | 5.993 | | 4.306 | |

% of control = (Test – Control)/Control × 100; Letters mean the significant differences between treatments according to Duncan's test
Data are the means ±SE (Standard error) of three replicates of 50 4th larvae each

***Determination of total proteins and acetyl cholinesterase enzyme activity.*** From the results in Table 4, it can be observed that all the tested synthesized compounds caused a decrease in total proteins; it was by -53.07%, -36.48, -24.21 and -18.24% lower than in the control corresponding to **16a**, **8**, **28** and **31b**, respectively. On the other hands, results indicated that all the tested synthesized compounds caused a remarkable increase in acetyl cholinesterase activity (Table 4), the enzyme activity of **16a** treated larvae reached its maximum level with (57.21% higher than in the control), and while **31b** caused the lowest remarkable increase in the enzyme activity (12.21% greater than in the control).

**Table 4 Total proteins and Acetyl cholinesterase activity in haemolymph of the 4th instar larvae *S. littoralis* (Boisd.) after 5 days of treatment with $LC_{50}$ of 16a, 8, 28 and 31b**

| Tested compounds | Total proteins (g/dl) | % of control | Acetyl cholinesterase (mU/ml) | % of control |
|---|---|---|---|---|
| **16a** | $2.83^{d}$±0.18 | -53.07 | $218^{a}$±2.65 | 57.21 |
| **8** | $3.83^{c}$±0.18 | -36.48 | $200.83^{b}$±0.80 | 44.83 |
| **28** | $4.57^{b}$±0.18 | -24.21 | $186.5^{c}$±2.48 | 34.49 |
| **31b** | $4.93^{b}$±0.09 | -18.24 | $155.6^{d}$±2.8 | 12.21 |
| **Control** | $6.03^{a}$±0.38 | | $138.67^{e}$±1.92 | |
| **LSD=0.05** | 0.703 | | 7.105 | |

% of control = (Test – Control)/Control × 100; Letters mean the significant differences between treatments according to Duncan's test
Data are the means ±SE (Standard error) of three replicates of 50 $4^{th}$ larvae each

***Histological studies***

It is evident that the highly toxic synthesized compounds, **16a, 8, 28 and 31b** administered to the 4th larvae of S. littoralis by feeding on castor bean leaves treated with $LC_{50}$ concentrations, resulted in some remarkable cytological changes in the cuticle, fat bodies, midgut and Malpighian tubules. After five days of treatment the signs appeared in the larval cuticle (Figs. 2-5), show histopathological changes compared to those of the control (Fig. 1), treatment with compound **16a** revealed increase in the thickness of the fibrous layer (Fig. 2), while administration of compound **28** indicated increase the thickness of fibrous layer, showing folded layer (Fig. 4).Also compound **31b** exhibited folded layer and degeneration in the thickness of the fibrous layer (Fig. 5). The histological examination in the larval midgut sections after 5 days of application with compound **16a** (Fig. 7) showed some fat bodies, which appeared to be dissolved as a result of the exposure, showing necrobiotic changes of the cells compared with the fat bodies of the control lar¬vae (Fig. 6). Administration with compound **8** showed sever necrosis of the cells (Fig. 8), and treatment with compound **28** showed increasing the number of fat cells most of them necrosed and pyknosis of the nucleous (Fig. 9), also the larvae feed on compound **31b** exhibited the fat body seems to be dissolved as a result of the treatment, showing necrosis

in some of fat cells (Fig. 10). The first noticeable histopathological signs appeared in the larval midgut after 5 days of application (Figs. 12-15), compared to that of the control (Fig. 11), some epithelial cells showed apparent histolysis and cytoplasmic vacuolation and some cells have pyknotic nuclei due to administration to compound **16a**. Also, the apical brush border of the epithelial cells was appeared to be destroyed (Fig. 12), similarly, treatment with compound **8** showed increase of number of goblet cells and necrosis in the columnar cells (Fig. 13), on the other hands, application by compound **28** caused the muscle fibers of treated individuals were separated from each other leaving a degenerated area in-between, showing sever necrosis (cell death) of cells only ruminant appeared and pyknotic cells of the basement membrane (Fig. 14). Treatment with compound **31b** caused the peritrophic membrane completely disappeared, and the regenerative cells were dissolved, showing necrosis of the cellular layers, destruction in the number of the columnar cells on the basement membrane (ruminant cells) and pyknosis of the cells appeared(Fig. 15). In addition, the Malpighian tubules of larvae exposed to compound **28** were highly affected after 5 days of application. The lumen of the Malpighian tubules appeared to be filled with secretion and the cells had pyknotic nuclei (Fig. 19) compared with those found in the control larvae (Fig. 16). Also treatment with newly synthesized heterocyclic compounds, **8** and **31b** revealednecro biotic changes and pyknosis of cells (Fig. 18, 20).

***Biological studies***

***Effects of the tested synthesized compounds on some biological properties of S. littoralis.***

The biological aspects of *S. littoralis* was investigated, thus, the newly moulted of $4^{th}$ instar larvae were left to feed on caster bean leaves treated with $LC_{25}$ of the most potent toxic

sulfonamido thiazoyl derivatives **16a**, **8**, **28** and **31b** for 48 h and then untreated leaves until pupation[23]. The main biological aspects were recorded and the results were represented in (Tables 5 and 6).

***Larval and Pupal duration.*** The results represented in (Table 5) indicated that all the tested compounds possess a significantly prolonged the larval duration which recorded as **16a** (23.67 days), **8** (19.33 days), **28** (16.00 days) and **31b** (14.67 days) compared to the control (13.33 days). On the contrary, the tested compounds decreased the pupal duration with a significantly differences between each other, which tabulated as **16a** (8.33 days) and **8** (9.67 days), while **28** and **31b** have no significantly difference among them (11, 11.33 days, respectively) compared to the untreated larvae (12.33 days).

***Pupal weight.*** From data tabulated in (Table 5), the same direction was observed on pupal weight, as the tested compounds possess a significant decrease of the pupal weight with a significantly differences between each other, where **16a** was the highest effective, recording (305.7 mg) followed by **8**, **28** (315.19, 323.85 mg, respectively) comparing to the control (346.24 mg), while **31b** was the least effective (331.02 mg) on pupal weight.

***% of Normal, Deformed pupae and Adult emergency***

With respect to the latent effects, the data in (Table 5), revealed that compounds **16a** and **8** were the most effective, recording (34.97, 14.40 and 70.88%) and (77.89, 13.57 and 67.01%), respectively, compared to the control group (97.27, 1.83 and 97.49%) to percentages of normal pupae, deformed pupae and adult emergence, respectively, followed by **28** (85.93, 6.83, 79.06%), and **31b** (93.29, 4.43, 80.24%).

***% of Fecundity and Egg hatchability***

Regarding to the data represented in (Table 6), number of eggs/female, percentage of fecundity and percentage of eggs hatchability, we observed that **16a** and **8** had a noticeably significant decrease of the mean numbers of eggs laid by adult female (fecundity), also eggs hatchability (fertility) was sharply decreased in the offspring generation after treatment of the parent 4$^{th}$ instar larvae with **16a** recording 405.0 eggs/female, 18.87% fecundity and 67.89% fertility followed by **8** (803.67 eggs/female, 37.38% fecundity and 61.08% fertility), compared to control group (2141.67 eggs/female, 100% fecundity and 97.63% fertility).**31b** was the least effective one giving (1720.0 eggs/female, 80.25% fecundity and 81.95% fertility), while compound **28** exhibited (1306.67 eggs/female, 60.97% fecundity and 75.25% fertility). The disorder in fecundity may be due to disfunction of maturation of an insect egg which depends on the materials that are produced by the ovary in suit which contains protein, lipids and carbohydrates, all of which required for embryonic structure[24].

***Adult longevity***

The obtained data in (Table 6) revealed that the tested synthetic sulfonamides bearing thiazole derivatives **164**, **8**, **28** and **31b** induced a significantly reduction of the adult longevity of both males and females, as **16a** was the most effective possessing highly significantly reduction of the adult longevity to average (4.55 and 7.14 days), followed by **8** (8.18 and 9.03 days), **28** (10.40 and 11.93 days), and **31b** (13.98 and 15.03 days) as compared to the control group (14.83 and 16.13 days), for male and female longevity, respectively.

**Table 5 Effects of the highly toxic newly synthesized compounds 16a, 8, 28 and 31b at their $LC_{25}$ values on some biological aspects of laboratory strain of the 4$^{th}$ instar larvae *S. littoralis* (Boisd.)**

| Tested compounds | $LC_{25}$ mg/L | Larval duration Days±SE | Pupal duration Days±SE | Pupal weight (mg)±SE | Normal pupae %±SE | Deformed pupae %±SE | Adult emergence %±SE |
|---|---|---|---|---|---|---|---|
| **16a** | 30.75 | $23.67^{a}$ ±0.33 | $8.33^{d}$ ±0.33 | $305.7^{e}$ ±0.40 | $34.97^{e}$ ±0.66 | $14.40^{a}$ ±0.32 | $70.88^{c}$ ±0.53 |
| **8** | 37.18 | $19.33^{b}$ ±0.33 | $9.67^{c}$ ±0.33 | $315.19^{d}$ ±0.18 | $77.89^{d}$ ±0.50 | $13.57^{a}$ ±0.35 | $67.01^{d}$ ±0.94 |
| **28** | 41.53 | $16.00^{c}$ ±0.00 | $11.00^{b}$ ±0.00 | $323.85^{c}$ ±0.19 | $85.93^{c}$ ±0.39 | $6.38^{b}$ ±0.32 | $79.06^{b}$ ±0.58 |
| **31b** | 49.93 | $14.67^{d}$ ±0.33 | $11.33^{b}$ ±0.33 | $331.02^{b}$ ±0.16 | $93.29^{b}$ ±0.85 | $4.43^{c}$ ±0.30 | $80.24^{b}$ ±0.38 |
| **Control** | | $13.33^{e}$ ±0.33 | $12.33^{a}$ ±0.33 | $346.24^{a}$ ±0.44 | $97.27^{a}$ ±0.37 | $1.83^{d}$ ±0.20 | $97.49^{a}$ ±0.64 |
| **LSD=0.05** | | 0.94 | 0.94 | 0.94 | 1.84 | 0.96 | 2.03 |

Letters mean the significant differences between treatments according to Duncan's test
SE = Standard error

**Table 6 Effects of the highly toxic newly synthesized compounds 16a, 8, 28 and 31b at their $LC_{25}$ values on fecundity, fertility and adult longevity for survived 4th instar larvae *S. littoralis* of laboratory strain (Boisd.)**

| Tested compounds | No. of eggs/female ±SE | Fecundity% ±SE | Egg hatchability% ±SE | Adult longevity Days±SE | |
|---|---|---|---|---|---|
| | | | | Male | Female |
| **16a** | $405^{e}$±18.93 | $18.87^{e}$±0.20 | $67.89^{d}$±0.20 | $4.55^{e}$±0.13 | $7.14^{e}$±0.18 |
| **8** | $803.67^{d}$±9.49 | $37.38^{d}$±0.1 | $61.08^{e}$±0.09 | $8.18^{d}$±0.04 | $9.03^{d}$±0.15 |
| **28** | $1306.67^{c}$±29.63 | $60.97^{c}$±0.04 | $75.25^{c}$±0.38 | $10.4^{c}$±0.06 | $11.93^{c}$±0.18 |
| **31b** | $1720^{b}$±10.41 | $80.25^{b}$±0.03 | $81.95^{b}$±0.25 | $13.98^{b}$±0.04 | $15.03^{b}$±0.15 |
| **Control** | $2141.67^{a}$±11.67 | $100^{a}$ | $97.63^{a}$±0.32 | $14.83^{a}$±0.09 | $16.13^{a}$±0.19 |
| **LSD=0.05** | 55.85 | 0.32 | 0.85 | 0.25 | 0.53 |

Letters mean the significant differences between treatments according to Duncan's test
SE = Standard error

## CONCLUSION

In this present work, it was thought worthwhile to build up some innovative bioactive polyfunctionallized substituted heterocyclic compounds incorporating a sulfonamide thiazole moiety of potential insecticidal efficacy against the cotton leafworm, *Spodoptera littoralis* under laboratory conditions. Moreover, estimation of the mode of action of the most potent tested insecticides by determination of biochemical parameters, (Enzymatic activity) such as Alk-p, ALT, AST, T. protein and Ach-E, in addition to, histological

studies. Furthermore, assessment of the latent effects of the tested compounds on some biological aspects such as larval and pupal duration, pupal weight, percentage of normal, deformed pupae and adult emergency, percentage of fecundity and egg hatchability, moreover, adult longevity with a hope to get better insecticidal agents slightly side effects. Regarding the determined $LC_{50}$ and $LC_{90}$ values, sulfonamides bearing thiazole moiety **16a**, **8**, **28**, **31b** and **7** showed the most potent toxic effects with $LC_{50}$ values of 49.04, 62.66, 78.62, 94.90 and 105.10 ppm, respectively, and toxicity index being 100, 78.26, 62.38, 51.68 and 46.66%, respectively. Also, it caused enzymatic disturbance either with increase ALT, AST and Ach-E activities or with decrease Alk-p and T. protein, in addition to a remarkable cytological changes in the cuticle, fat bodies, midgut and Malpighian tubules. Furthermore, the latent effects of the tested compounds according to sublethal concentrations against $4^{th}$ instar larvae of laboratory strain were significantly decreased in pupal duration, pupal weight, percentage of normal pupae, adult emergence, fecundity, fertility and adult longevity compared to the control, while these compounds prolonged significantly larval duration and percentage of malformed pupae with significant differences among them.

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