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A FACILE GREEN SYNTHESIS AND ANTI-CANCER ACTIVITY OF *BIS*-ARYLHYDRAZONONITRILES, TRIAZOLO[5,1-*c*][1,2,4]TRIAZINE, AND 1,3,4-THIADIAZOLINES

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Abstract – Coupling of 2-cyanoacetyl-1-methyl-1*H*-pyrrole (**1**) with diazonium salts of 1,4-benzenediamine (**2**), 2,6-dichlorobenzene-1,4-diamine (**4**), and benzidine (**6**) afforded *bis*-arylhydrazononitriles **3**, **5**, and **7**, respectively. Also, coupling of **1** with [1,2,4]triazole-3-diazonium sulfate (**8**) gave the respective [1,2,4]triazolo[5,1-*c*][1,2,4]triazine derivative **11**. On the other hands, treatment of 2-[(1-methyl-1*H*-pyrrol-2-yl)carbonyl]-3-mercapto-3-(phenylamino)acrylonitrile (**12**) with hydrazonoyl chlorides **13a-h** in dioxane, in the presence of chitosan as eco-friendly heterogeneous basic catalyst, under microwave irradiation furnished 1,3,4-thiadiazolines **16a-h**, incorporating pyrrole moiety. The anti-cancer activities of the synthesized products were determined against the colon carcinoma (HCT), human laryngeal carcinoma (Hep-2), human medulloblastoma (Daoy), human breast adenocarcinoma (MCF-7), and human colon adenocarcinoma (WiDr) cell lines.

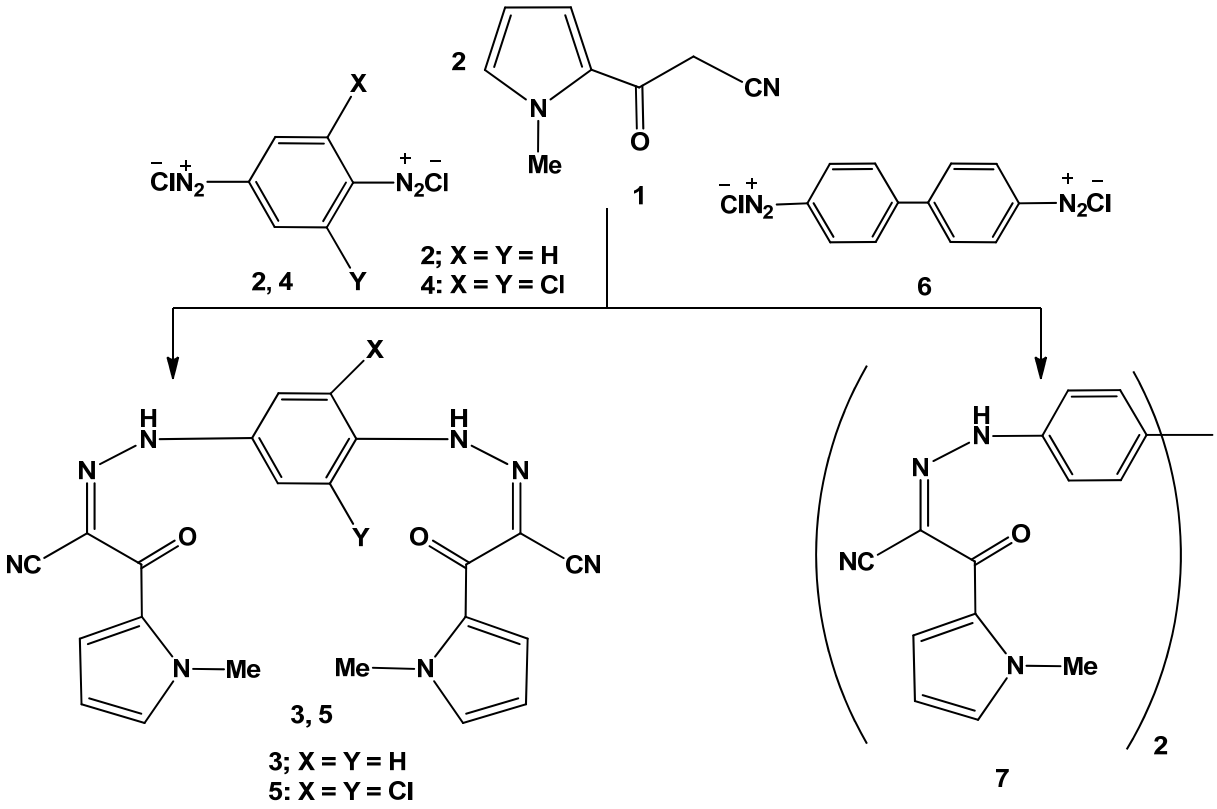
Arylazo compounds have multifarious industrial applications such as printing, electronic photography, liquid crystal displays, laser technology,[1] and dye manufacture.[2] Also, they are widely used in chromoionophores because they could exhibit substantial color changes observable by the naked eyes

upon complexation with metal ions[3] such as Ca^{+2}, and Pb^{+2}. Moreover, *bis*-arylazo compounds were reported to be used as a ratiometric and specific chromogenic sensor for Hg^{+2} in polar protic solvent.[4] The rich chemistry of azo compounds is also associated with several important biological activities such as antimicrobial,[5] antifungal,[6] anticonvulsant,[7] antioxidant, and antitumor.[8] Recently, the synthesis of thiadiazoline derivatives[9] has attracted considerable attention because these compounds have a wide range of biological properties. Thiadiazolines could be act as anthelmintics,[10] antihypertensive,[11] antitumor,[12] analgesic,[13] and antimicrobial agents.[14] In view of the immense importance associated with arylazo and thiadiazolines and in continuation with our work related to the synthesis and evaluation the biological activities of aza-heterocycles,[15-18] it was envisaged to undertake the synthesis and evaluation of the anti-cancer activity of a series of novel *bis*-arylazo and 1,3,4-thiadiazoline compounds. In this context, we could successfully utilized chitosan as efficient, eco-friendly heterogeneous basic catalyst under microwave irradiation to afford a novel environmentally benign route for synthesis of 1,3,4-thiadiazoline compounds.

2-Cyanoacetyl-1-methyl-1*H*-pyrrole (**1**),[19] prepared via cyanoacetylation of 1-methyl-1*H*-pyrrole in the presence of catalytic amount of $InCl_3$, was coupled with diazonium salts of appropriate aromatic amines [1,4-benzenediamine (**2**), 2,6-dichlorobenzene-1,4-diamine (**4**), and benzidine (**6**)] in molar ratio (2:1) in ethanol in the presence of sodium acetate to afford the respective *bis*-hydrazones [**3**, **5**, and **7**, respectively] (Scheme 1). The structures of the products were established on the basis of their elemental analyses and spectral data (IR, ^{1}H NMR, ^{13}C NMR, MS).

Hydrazones can be formulated in different possible tautomeric structures, namely keto-hydrazone (**A**), azo-enol (**B**), and CH-azo tautomer (**C**) (Figure 1). The ^{1}H NMR spectra of the studied compounds showed signal in the region of δ = 13.83-13.92 ppm assignable to hydrazone proton (-CH=N-NH-).[20] Signal at δ = 5.27 ppm,[21] which is characteristic for the CH protons of CH-azo form, was not detected. Also, IR spectra did not reveal broad signal at 3434 cm^{-1}, which is characteristic for OH of azo-enol form.[22] These spectroscopic analyses allow to rule out the azo-enol form (**B**), CH-azo form (**C**), and confirm keto-hydrazone form (**A**) (Figure 1).

Keto-hydrazone tautomer (**A**) can be existed in two geometric structures (*E* and *Z*). IR spectra of compounds **3**, **5**, and **7** showed a shift of the CO bond stretching to lower wave number due to both conjugation with C=N and formation of a hydrogen bond with the NH group. Furthermore, the downfield shift of hydrazone proton signal (δ = 13.83-13.92 ppm) in ^{1}H NMR is characteristic for the formation of an intramolecular hydrogen bond of the NH proton for structure (**A**). These results confirm only the presence of *E*- form.[23]



Scheme 1

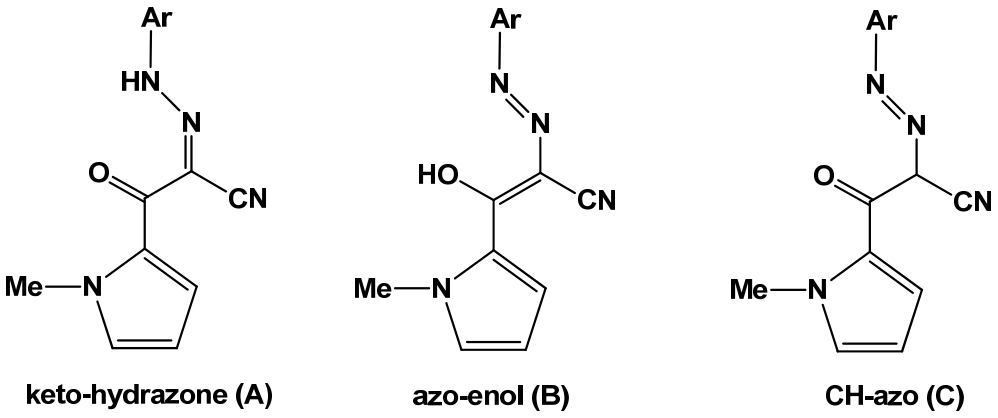
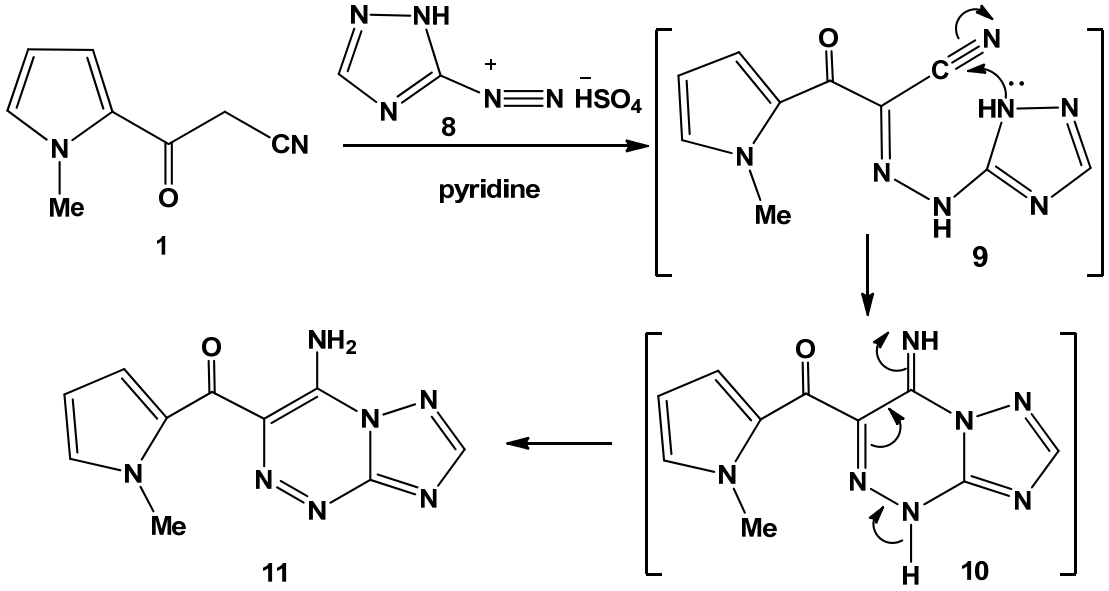


Figure 1

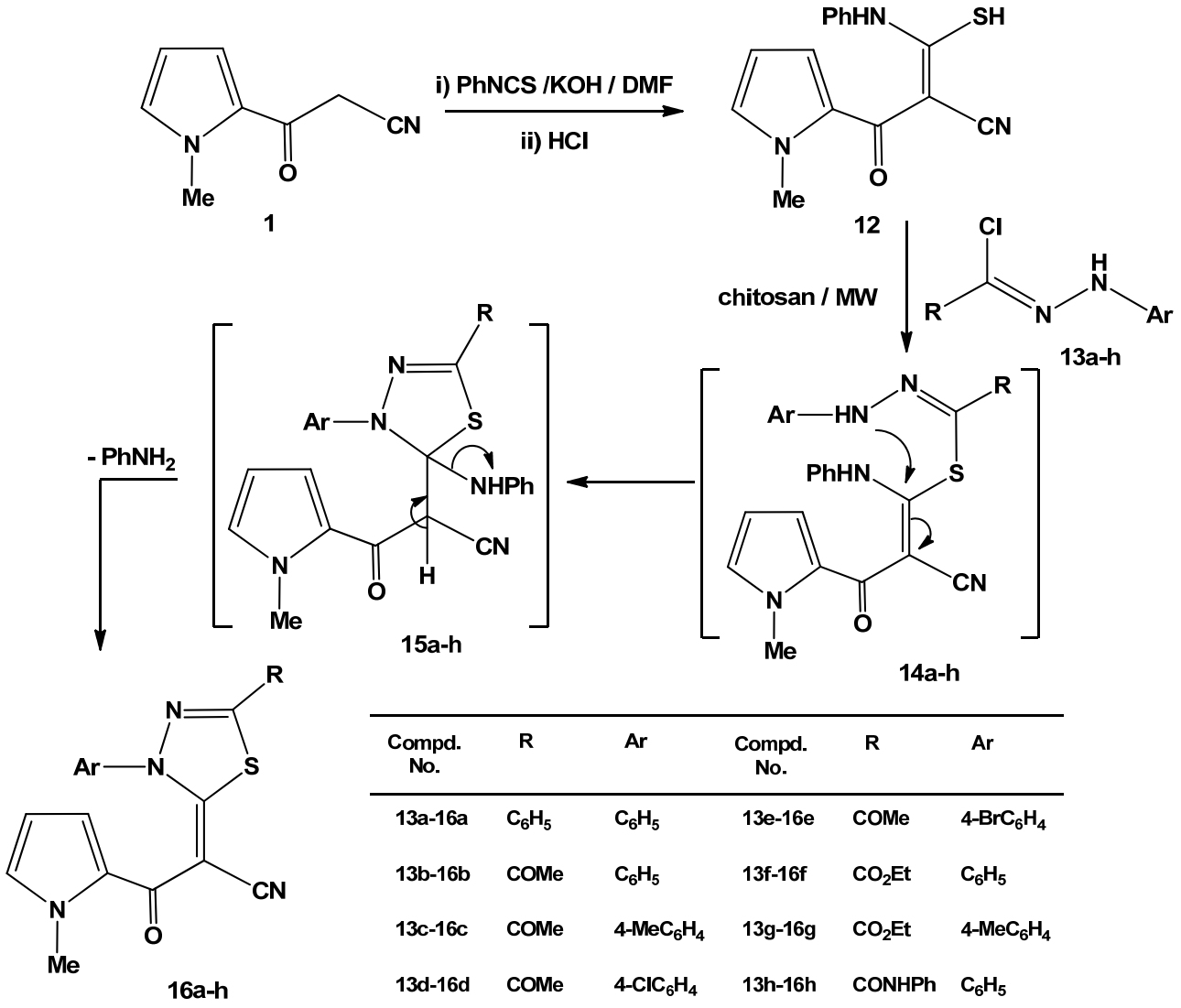
In continuation of our interest in the synthesis of bridged-head nitrogen heterocyclic systems,[24] we have found that diazotized heterocyclic amines are excellent building blocks for the synthesis of the target compounds. Thus, coupling of 2-cyanoacetyl-1-methyl-1*H*-pyrrole (**1**) with [1,2,4]triazole-3-diazonium sulfate (**8**) in pyridine at 0-5 ^{o}C afforded 4-amino-3-[(1-methyl-1*H*-pyrrol-2-yl)carbonyl][1,2,4]triazolo-[5,1-*c*][1,2,4]triazine (**11**)[25] (Scheme 2). The structure of the isolated product was elaborated by its elemental analyses and spectral data. IR spectrum of **11** showed absorption bands at ν = 3389, 3221 cm^{-1} assignable to NH_2 group besides carbonyl absorption band at ν = 1687 cm^{-1}. Also, its ^{1}H NMR

spectrum revealed a singlet signal at δ 3.90 ppm (D_2O-exchangeable) due to NH_2 protons. The mechanism of formation of product **11** seems to start *via* initial coupling of diazonium salt on active methylene group in compound **1** to form the respective non-isolable hydrazone intermediate **9** which then underwent intramolecular cyclization followed by aromatization to give the isolated product **11** (cf. Scheme 2).



Scheme 2

Next, our study was extended to explore a new synthetic approach to 1,3,4-thiadiazoline derivatives via green chemical techniques. Thus, stirring of 2-cyanoacetyl-1-methyl-1*H*-pyrrole (**1**) with phenyl isothiocyante in dimethylformamide, in the presence of potassium hydroxide, at room temperature gave the respective 2-[(1-methyl-1*H*-pyrrol-2-yl)carbonyl]-3-mercapto-3-(phenylamino)acrylonitrile (**12**) as previously mentioned[26] (Scheme 3). Ttreatment of equimolar amounts of **12** with hydrazonoyl chlorides **13a-h** in dioxane, in the presence of catalytic amount of chitosan (10% wt), under microwave irradiation resulted in the formation of 1,3,4-thiadiazolines **16a-h**, with reaction times from 3 to 5 min at 300 W of power and temperatures of 150-160 ^{o}C (Scheme 3). The reaction products were easily obtained in high yields (90-94%). The spectroscopic data are consistent with structures **16a-h**. These compounds showed the stretching bands of the C≡N group at 2188-2200 cm^{-1} while the carbonyl bands appeared at 1698-1705 cm^{-1}. ^{1}H NMR revealed a singlet signal at δ 3.47-3.78 ppm corresponding to (N-CH_3) protons and another multiplet signals at δ 7.12-8.16 ppm corresponding to aromatic protons. In the ^{13}C NMR spectrum the presence of a signal at δ 65.81-65.93 ppm, corresponding to vinylic carbon adjacent to nitrile group, and another signal at δ 164.30-165.42 ppm, corresponding to thiadiazoline C-2, confirms that such structure corresponds to 1,3,4-thiadiazol-2(3*H*)-ylidene derivatives.[27]



Compd. No.	R	Ar	Compd. No.	R	Ar
13a-16a	C_6H_5	C_6H_5	13e-16e	COMe	4-BrC_6H_4
13b-16b	COMe	C_6H_5	13f-16f	CO_2Et	C_6H_5
13c-16c	COMe	4-MeC_6H_4	13g-16g	CO_2Et	4-MeC_6H_4
13d-16d	COMe	4-ClC_6H_4	13h-16h	CONHPh	C_6H_5

Scheme 3

In Scheme 3, we postulate a plausible mechanism for such reactions. Firstly, nucleophilic displacement of (SH) group with concurrent elimination of hydrogen chloride afforded intermediates **14a-h**. Intramolecular Michael type addition of (NH) group to vinylic double bond gave non-isolable Michael adducts **15a-h**. Finally, elimination of aniline molecule from intermediates **15a-h** gave the isolated products **16a-h**.

The anti-cancer activities of the synthesized compounds **3**, **5**, **7**, **11**, **16a**, **16b**, **16f** and **16h** were determined against the colon carcinoma (HCT), human laryngeal carcinoma (Hep-2), human medulloblastoma (Daoy), human breast adenocarcinoma (MCF-7), and human colon adenocarcinoma (WiDr) cell lines. Data generated were used to plot a dose response curve of which the concentration of test compounds required to kill 50% of cell population (IC_{50}) was determined. Cytotoxic activity was expressed as the mean IC_{50} of three independent experiments (Table 1).

Table 1. Cytotoxic activities of compounds **3, 5, 7, 11, 16a, 16b, 16f,** and **16h** against tumor cell lines

Compound No.	IC_{50} (μg / mL)				
	HCT	**Hep-2**	**Daoy**	**MCF-7**	**WiDr**
3	18.60	7.03	8.14	7.64	6.87
5	20.41	10.32	11.34	11.74	10.02
7	24.03	14.34	14.56	14.62	13.05
11	> 50	25.31	26.04	26.07	27.48
16a	36.21	17.32	17.05	16.98	17.55
16b	32.87	16.54	16.34	16.11	17.28
16f	33.21	15.68	15.97	15.14	15.78
16h	38.42	19.34	18.91	17.62	19.16

Cell lines; HCT (colon carcinoma), Hep-2 (human laryngeal carcinoma), Daoy (human medulloblastoma), MCF-7 (human breast adenocarcinoma), WiDr (human colon adenocarcinoma)

The results revealed that, *bis*-arylhydrazononitriles **3**, **5**, and **7** have highest cytotoxicity values compared to triazolotriazine **11** and 1,3,4-thiadiazolines **16**, which could be attributed to formation of azo-hydrazo isomers.[28] Also, 1,3,4-thiadiazolines **16a**, **16b**, **16f**, and **16h** have higher cytotoxicity values than triazolotriazine **11**, which could be attributed to size of heterocycles, number of nitrogen atoms in azoles and azines rings, and the nature of fusion of azoles or azines.[28]

Novel series of *bis*-arylhydrazononitriles, triazolotriazine, and 1,3,4-thiadiazolines, bearing pyrrole moiety were synthesized and evaluated for their anticancer activities. Most of the newly synthesized products revealed moderate anticancer activity against colon carcinoma (HCT), human laryngeal carcinoma (Hep-2), human medulloblastoma (Daoy), human breast adenocarcinoma (MCF-7), and human colon adenocarcinoma (WiDr) cell lines.

EXPERIMENTAL

General

Melting points were measured on a Gallenkamp melting point apparatus (Weiss-Gallenkamp, London, UK). IR spectra were recorded in potassium bromide discs on Pye Unicam SP 3300 and Shimadzu FTIR 8101 PC infrared spectrophotometers. NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer operating at 400 MHz (^{1}H NMR) or 100 MHz (^{13}C NMR) and run in deuterated

dimethylsulfoxide (DMSO-d_6). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCeMS-QP1000 EX mass spectrometer at 70 eV. Elemental analyses were measured by using a German made Elementar vario LIII CHNS analyzer. Microwave experiments were carried out using CEM Discover Labmate microwave apparatus (300 W with Chem. Driver Software). The cytotoxic evaluation of some selected examples was carried out in the Regional Center for Mycology and Biotechnology of Al-Azhar University, Cairo, Egypt. 2-Cyanoacetyl-1-methyl-1*H*-pyrrole (**1**)[19] and hydrazonoyl halides[29,30] were prepared as previously reported in the respective literature.

Coupling of 2-cyanoacetyl-1-methyl-1H-pyrrole (1) with the appropriate diazonium salt of aromatic amines

General procedure:

To a cold solution of 2-cyanoacetyl-1-methyl-1*H*-pyrrole (**1**) (0.296 g, 2 mmol) in EtOH (20 mL), containing sodium acetate trihydrate (0.138 g, 1 mmol), was added the appropriate diazonium salt of aromatic amines [1,4-benzenediamine (**2**) (0.108 g, 1 mmol), 2,6-dichlorobenzene-1,4-diamine (**4**) (0.177 g, 1 mmol), and benzidine (**6**) (0.184 g, 1 mmol)] portion wise over a period of 30 min. After stirring for further 2 h, at 0-5 ^{o}C, the reaction mixture was diluted with water and extracted with $CHCl_3$. After evaporation of solvent, the crude product was purified by passing through column chromatography on silica gel using hexane/EtOAc (5:1) as an eluent.

N',N''-(1,4-Phenylene)bis[2-(1-methyl-1H-pyrrol-2-yl)-2-oxoacetohydrazonoyl cyanide] (3).

Red solid, (0.26 g, 61%), mp 230-232 ^{o}C; IR (KBr) υ 3281, 3154 (2NH), 2249, 2253 (2C≡N), 1691, 1687 (2C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ = 3.82 (s, 6H, 2 N-CH_3), 6.35-7.79 (m, 10H, Ar-H), 13.85 (s, 2H, D_2O-exchangeable, 2NH) ppm; ^{13}C NMR (DMSO-d_6) δ = 32.62 (2CH_3), 116.88 (2C≡N), 110.18, 121.72, 123.54, 129.43, 132.18, 148.22 (Ar-C), 178.12 (2C=O); MS, *m/z* (%) 426 (M^+, 7), 400 (40), 318 (100). *Anal.* Calcd for $C_{22}H_{18}N_8O_2$ (426.16): C, 61.96; H, 4.25; N, 26.28. Found: C, 62.07; H, 4.38; N, 26.40%.

N',N''-(2,6-Dichloro-1,4-phenylene)bis[2-(1-methyl-1H-pyrrol-2-yl)-2-oxoacetohydrazonoyl cyanide] (5).

Dark red solid, (0.35 g, 71%), mp 249-251 ^{o}C; IR (KBr) υ 3265, 3158 (2NH), 2248, 2259 (2C≡N), 1698, 1685 (2C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ = 3.80 (s, 6H, 2 N-CH_3), 6.77-7.99 (m, 8H, Ar-H), 13.83 (s, 2H, D_2O-exchangeable, 2NH) ppm; ^{13}C NMR (DMSO-d_6) δ = 32.52 (2CH_3), 116.68 (2C≡N), 112.38, 122.21, 123.54, 123.98, 126.87, 129.43, 132.18, 136.37, 148.31 (Ar-C), 179.10 (2C=O); MS, *m/z* (%) 498 (M^++4, 4), 496 (M^++2, 12), 494 (M^+, 40), 468 (30), 386 (100). *Anal*. Calcd for $C_{22}H_{16}Cl_2N_8O_2$ (494.08):

C, 53.35; H, 3.26; N, 22.62. Found: C, 53.27; H, 3.12; N, 22.43%.

N',N''-(Biphenyl-4,4'-diyl)bis[2-(1-methyl-1H-pyrrol-2-yl)-2-oxoacetohydrazonoyl cyanide] (7).

Red solid, (0.35 g, 70%), mp 205-207 ^{o}C; IR (KBr) υ 3251, 3119 (2NH), 2243, 2250 (2C≡N), 1693, 1685 (2C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ = 3.85 (s, 6H, 2 N-CH$_3$), 6.68-7.82 (m, 14H, Ar-H), 13.92 (s, 2H, D$_2$O-exchangeable, 2NH) ppm; ^{13}C NMR (DMSO-d_6) δ = 33.12 (2CH$_3$), 117.28 (2C≡N), 112.38, 120.54, 121.72, 123.54, 128.13, 129.41, 132.18, 142.55, 148.22 (Ar-C), 179.18 (2C=O); MS, *m/z* (%) 502 (M^{+}, 20), 476 (40), 394 (100). *Anal*. Calcd for $C_{28}H_{22}N_8O_2$ (502.19): C, 66.92; H, 4.41; N, 22.30. Found: C, 67.07; H, 4.34; N, 22.42%.

Coupling of 2-cyanoacetyl-1-methyl-1H-pyrrole (1) with diazonium salt of heterocyclic amine.

To a cold solution of 2-cyanoacetyl-1-methyl-1*H*-pyrrole (**1**) (0.148 g, 1 mmol) in pyridine (20 mL), was added the diazonium salt of 3-amino-[1,2,4]triazole (**8**) (0.084 g, 1mmol), prepared according to literature procedures.[31] After complete addition of the diazonium salt, the reaction mixture was stirred for a further 2 h in an ice bath, and then poured onto ice/HCl mixture. The solid precipitated was filtered off, washed with water, dried and crystallized from EtOH to give the respective product **11**.

4-Amino-3-[(1-methyl-1H-pyrrol-2-yl)carbonyl][1,2,4]triazolo[5,1-c][1,2,4]triazine (11).

Yellow solid, (0.16 g, 66%), mp 154-156 ^{o}C; IR (KBr) υ 3389, 3221 (NH$_2$), 1687 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.17 (s, 3H, N-CH$_3$), 3.90 (br, 2H, D$_2$O-exchangeable, NH$_2$), 6.96-7.32 (m, 3H, Ar-H), 8.59 (s, 1H, triazole-H) ppm; MS, *m/z* (%) 243 (M^{+}, 15), 163 (100), 135 (21). *Anal.* Calcd for $C_{10}H_9N_7O$ (243.09): C, 49.38; H, 3.73; N, 40.31. Found: C, 49.57; H, 3.88; N, 40.57%.

Synthesis of 2-[(1-methyl-1H-pyrrol-2-yl)carbonyl]-3-mercapto-3-(phenylamino)acrylonitrile (12).

To a stirred solution of potassium hydroxide (0.56 g, 10 mmol) in DMF (30 mL) was added 2-cyanoacetyl-1-methyl-1*H*-pyrrole (**3**) (1.48 g, 10 mmol). After stirring for 30 min, phenyl isothiocyanate (1.35 g, 10 mmol) was added to the resulting mixture. Stirring was continued overnight. The reaction mixture was acidified with HCl and the solid product was filtered off, washed with water and dried. Recrystallization from EtOH gave white solid (1.66 g, 70%), mp 156 ^{o}C; IR (KBr) υ 3246 (NH), 2201 (C≡N), 1705 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.45 (s, 3H, N-CH$_3$), 7.15-7.59 (m, 8H, Ar-H), 10.23 (s, 1H, D$_2$O-exchangeable, NH), 11.58 (s, 1H, SH) ppm; MS, *m/z* (%) 283 (M^{+}, 7), 266 (17), 108 (100), 93 (85). *Anal*. Calcd for $C_{15}H_{13}N_3OS$ (283.08): C, 63.58; H, 4.62; N, 14.83; S, 11.32. Found: C, 63.81; H, 4.78; N, 14.99; S, 11.53%.

Reactions of 2-[(1-methyl-1H-pyrrol-2-yl)carbonyl]-3-mercapto-3-(phenylamino)acrylonitrile (12) with hydrazonoyl chlorides under microwave irradiation.

To a solution of 2-[(1-methyl-1*H*-pyrrol-2-yl)carbonyl]-3-mercapto-3-(phenylamino)acrylonitrile (**12**) (0.283 g, 1 mmol) and the appropriate hydrazonoyl chlorides **13a-h** (1 mmol of each) in dioxane (10 mL) was added chitosan (0.1 g) at room temperature. The reaction mixture was irradiated under constant pressure (11.2 Bar, 150-160 ^{o}C) for 3-5 min at a power of 300 W. The hot solution was filtered to remove chitosan. After cooling, dil. HCl was added till pH became acidic, and the solid product was collected and recrystallized from EtOH or EtOH/DMF mixture to give products **16a-h**. The physical constants together with the spectral data of **16a-h** are listed below.

2-[(3,5-Diphenyl-1,3,4-thiadiazol-2(3H)-ylidene)]-3-(1-methyl-1H-pyrrol-2-yl)-3-oxopropanenitrile (16a).

Yellow solid (0.35 g, 91%), mp 272 ^{o}C; IR (KBr) υ 2188 (C≡N), 1703 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.47 (s, 3H, N-CH$_3$), 7.12-7.86 (m, 13H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 36.25, 65.82, 115.98, 119.35, 123.31, 126.12, 126.53, 127.90, 128.23, 129.16, 129.77, 130.04, 131.16, 133.42, 136.02, 156.77, 165.42, 178.13 ppm; MS, *m/z* (%) 384 (M^{+}, 50), 194 (16), 108 (100), 77 (50), 53 (75). *Anal*. Calcd for $C_{22}H_{16}N_4OS$ (384.10): C, 68.73; H, 4.19; N, 14.57; S, 8.34. Found: C, 68.46; H, 4.12; N, 14.49; S, 8.19%.

2-[(5-Acetyl-3-phenyl-1,3,4-thiadiazol-2(3H)-ylidene)]-3-(1-methyl-1H-pyrrol-2-yl)-3-oxopropane-nitrile (16b).

Yellow solid (0.32 g, 92%), mp 302 ^{o}C; IR (KBr) υ 2195 (C≡N), 1704, 1698 (2C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.68 (s, 3H, COCH$_3$), 3.68 (s, 3H, N-CH$_3$), 7.16-7.83 (m, 8H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 26.73, 36.15, 65.86, 116.08, 119.11, 122.71, 125.82, 126.53, 127.93, 129.33, 133.12, 136.02, 159.78, 165.32, 178.33, 191.77 ppm; MS, *m/z* (%) 350 (M^{+}, 20), 324 (16), 307 (70), 242 (100), 77 (50). *Anal*. Calcd for $C_{18}H_{14}N_4O_2S$ (350.08): C, 61.70; H, 4.03; N, 15.99; S, 9.15. Found: C, 61.55; H, 3.82; N, 15.69; S, 9.03%.

2-[(5-Acetyl-3-(4-methylphenyl)-1,3,4-thiadiazol-2(3H)-ylidene)]-3-(1-methyl-1H-pyrrol-2-yl)-3-oxo-propanenitrile (16c).

Yellow solid (0.33 g, 90%), mp 293 ^{o}C; IR (KBr) υ 2192 (C≡N), 1705, 1693 (2C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.39 (s, 3H, Ar-CH$_3$), 2.64 (s, 3H, COCH$_3$), 3.75 (s, 3H, N-CH$_3$), 7.13-7.80 (m, 7H, Ar-H) ppm; MS, *m/z* (%) 364 (M^{+}, 50), 338 (16), 321 (30), 256 (10), 108 (100). *Anal*. Calcd for $C_{19}H_{16}N_4O_2S$ (364.10): C, 62.62; H, 4.43; N, 15.37; S, 8.80. Found: C, 62.35; H, 4.13; N, 15.60; S, 8.58%.

2-[(5-Acetyl-3-(4-chlorophenyl)-1,3,4-thiadiazol-2(3H)-ylidene)]-3-(1-methyl-1H-pyrrol-2-yl)-3-oxo-propanenitrile (16d).

Yellow solid (0.35 g, 90%), mp 237 ^{o}C; IR (KBr) υ 2194 (C≡N), 1704, 1695 (2C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.67 (s, 3H, $COCH_3$), 3.69 (s, 3H, N-CH_3), 7.18-7.89 (m, 7H, Ar-H) ppm; MS, *m/z* (%) 386 (M^++2, 7), 384 (M^+, 25), 341 (16), 276 (40), 108 (100), 53 (50). *Anal*. Calcd for $C_{18}H_{13}ClN_4O_2S$ (384.04): C, 56.18; H, 3.40; N, 14.56; S, 8.33. Found: C, 56.37; H, 3.53; N, 14.68; S, 8.51%.

2-[(5-Acetyl-3-(4-bromophenyl)-1,3,4-thiadiazol-2(3H)-ylidene)]-3-(1-methyl-1H-pyrrol-2-yl)-3-oxo-propanenitrile (16e).

Yellow solid (0.39 g, 91%), mp 249 ^{o}C; IR (KBr) υ 2193 (C≡N), 1705, 1692 (2C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.63 (s, 3H, $COCH_3$), 3.66 (s, 3H, N-CH_3), 7.15-7.78 (m, 7H, Ar-H) ppm; MS, *m/z* (%) 430 (M^++2, 20), 428 (M^+, 22), 385 (40), 319 (50), 108 (100), 53 (50). *Anal*. Calcd for $C_{18}H_{13}BrN_4O_2S$ (427.99): C, 50.36; H, 3.05; N, 13.05; S, 7.47. Found: C, 50.18; H, 3.17; N, 13.18; S, 7.61%.

Ethyl 5-[1-cyano-2-(1-methyl-1H-pyrrol-2-yl)-2-oxoethylidene)]-4-phenyl-4,5-dihydro-1,3,4-thiadiazole-2-carboxylate (16f).

Yellow solid (0.35 g, 93%), mp 218 ^{o}C; IR (KBr) υ 2200 (C≡N), 1714, 1698 (2C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.32 (t, 3H, $CH_2\underline{CH_3}$), 3.74 (s, 3H, N-CH_3), 4.53 (q, 2H, $\underline{CH_2}CH_3$), 7.17-7.81 (m, 8H, Ar-H) ppm; ^{13}C NMR (DMSO-d_6) δ 16.73, 36.11, 58.31, 65.93, 115.98, 119.43, 122.78, 124.82, 126.76, 128.13, 130.13, 132.98, 136.11, 159.88, 164.30, 178.33, 190.77 ppm; MS, *m/z* (%) 380 (M^+, 5), 307 (70), 272 (30), 108 (100), 77 (50). *Anal*. Calcd for $C_{19}H_{16}N_4O_3S$ (380.09): C, 59.99; H, 4.24; N, 14.73; S, 8.43. Found: C, 60.15; H, 4.42; N, 14.69; S, 8.63%.

Ethyl 5-[1-cyano-2-(1-methyl-1H-pyrrol-2-yl)-2-oxoethylidene)]-4-(4-methylphenyl)-4,5-dihydro-1,3,4-thiadiazole-2-carboxylate (16g).

Yellow solid (0.35 g, 90%), mp 246 ^{o}C; IR (KBr) υ 2198 (C≡N), 1718, 1700 (2C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.35 (t, 3H, $CH_2\underline{CH_3}$), 2.42 (s, 3H, Ar-CH_3), 3.78 (s, 3H, N-CH_3), 4.51 (q, 2H, $\underline{CH_2}CH_3$), 7.12-7.87 (m, 7H, Ar-H) ppm; MS, *m/z* (%) 394 (M^+, 15), 321 (40), 286 (30), 108 (100), 91 (20). *Anal*. Calcd for $C_{20}H_{18}N_4O_3S$ (394.11): C, 60.90; H, 4.60; N, 14.20; S, 8.13. Found: C, 60.85; H, 4.40; N, 14.29; S, 8.23%.

5-[(1-Cyano-2-(1-methyl-1H-pyrrol-2-yl)-2-oxoethylidene)]-N,4-diphenyl-4,5-dihydro-1,3,4-thiadiazole-2-carboxamide (16h).

Yellow solid (0.39 g, 91%), mp 226 ^{o}C; IR (KBr) υ 2193 (C≡N), 1704, 1665 (2C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.49 (s, 3H, N-CH_3), 7.14-8.16 (m, 13H, Ar-H), 11.14 (s, 1H, D_2O exchangeable, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 36.27, 65.81, 116.04, 119.33, 123.31, 126.17, 126.58, 127.88, 128.28, 129.19, 129.78, 130.54, 131.86, 133.42, 136.11, 156.77, 165.41, 168.19, 178.13 ppm; MS, *m/z* (%) 427 (M^+, 40), 319 (40), 307 (50), 108 (10), 77 (30), 69 (100). *Anal*. Calcd for $C_{23}H_{17}N_5O_2S$ (427.11): C, 64.62; H, 4.01; N, 16.38; S, 7.50. Found: C, 64.46; H, 4.12; N, 16.49; S, 7.39%.

Cytotoxic activity

Potential cytotoxicity of the compounds was tested using the method of Skehan *et al.*[32] using Sulfo-Rhodamine-B stain (SRB). Cells were plated in 96-multiwill plates (10^4 cells/well) for 24 h before treatment with the tested compound to allow attachment of cell to the wall of the plate. Different concentrations of the compound under test (0, 1.56, 3.125, 6.25, 12.5, 25, and 50 µg/mL) were added to the cell monolayer in triplicate wells individual dose, monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained with SRB stain, excess stain was washed with acetic acid and attached stain was recovered with *tris*-EDTA buffer, color intensity was measured in an ELISA reader.

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REFERENCES

1. G. S. Uscumlic, D. Z. Mijin, N. V. Valentic, V. V. Vajs, and B. M. Susic, *Chem. Phys. Lett.,* 2004, **397**, 148.
2. Y. M. ElKholy and S. A. Abd El-Hafiz, *Pigment Resin Technol.*, 1994, **23**, 3.
3. K. C. Chang, I. H. Su, G. H. Lee, and W. S. Chung, *Tetrahedron Lett.*, 2007, **48**, 7274.
4. N. J. Wang, C. M. Sun, and W. S. Chung, *Tetrahedron*, 2011, **67**, 8131.
5. B. P. Patel, H. S. Patel, and P. J. Shah, *Bulg. Chem. Commun.*, 2010, **42**, 274.
6. H. Xu and X. Zeng, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 4193.
7. S. G. Kucukguzel, S. Rollas, H. Erdeniz, M. Kiraz, A. C. Ekinci, and A. Vidin, *Eur. J. Med. Chem.*, 2000, **35**, 761.
8. T. A. Farghaly and M. M. Abdalla, *Bioorg. Med. Chem.*, 2009, **17**, 8012.
9. D. J. Wilkins, "Comprehensive Heterocyclic Chemistry III" Elsevier, Camelford, UK, 2008, p. 467.
10. X. C. Wang, L. Zheng, Z. J. Quang, and D. J. Xu, *Synth. Commun.*, 2003, **33**, 2891.

11. C. H. Oh, H. W. Cho, D. Baek, and J. H. Cho, *Eur. J. Med. Chem.*, 2002, **37**, 734.
12. M. A. Ilies, D. Vullo, J. Pastorek, A. Scozzafava, M. Ilies, M. T. Caproiu, S. Pastorokova, and C. T. Supuran, *J. Med. Chem.*, 2003, **46**, 2187.
13. M. Amir and K. Shikha, *Eur. J. Med. Chem.*, 2004, **39**, 535.
14. N. Demirbas, A. S. Karaoglu, A. Demirbas, and K. Sancak, *Eur. J. Med. Chem.*, 2004, **39**, 793.
15. K. D. Khalil, H. M. Al-Matar, D. M. Al-Dorri, and M. H. Elnagdi, *Tetrahedron*, 2009, **65**, 9421.
16. S. M. Gomha and K. D. Khalil, *Molecules*, 2012, **17**, 9335.
17. S. M. Gomha, S. M. Riyadh, I. M. Abbas, and M. A. Bauomi, *Heterocycles*, 2013, **87**, 341.
18. S. M. Riyadh and T. A. Farghaly, *Tetrahedron*, 2012, **68**, 9056.
19. H. M. Al-Matar, A. Y. Adam, K. D. Khalil, and M. H. Elnagdi, *ARKIVOC*, 2012, (**vi**), 1.
20. R. Brehme, D. Enders, R. Fernandez, and J. M. Lassaletta, *Eur. J. Org. Chem*., 2007, 5629.
21. J. G. Schantl and P. Hebeisen, *Tetrahedron,* 1990, **46**, 395.
22. E. O. M. Rufchahi and A. G. Gilani, *Dyes Pigments*, 2012, **95**, 632.
23. A. S. Shawali and M. A. El-Galil, *Tetrahedron,* 1971, **27**, 4305.
24. S. M. Riyadh, *Molecules*, 2011, **16**, 1834.
25. A. M. Farag, K. M. Dawood, and Z. E. Kandeel, *Tetrahedron*, 1996, **52**, 7893.
26. S. A. F. Rostom, I. M. El-Ashmawy, H. A. Abd El Razik, M. H. Badr, and H. M. A. Ashour, *Bioorg. Med. Chem.,* 2009, **17**, 882.
27. M. A. A. Radwan, E. A. Ragab, N. M. Sabry, and S. M. El-Shenawy, *Bioorg. Med. Chem.,* 2007, **15**, 3832.
28. A. El-Shafei, A. A. Fadda, A. M. Khalil, T. A. E. Ameen, and F. A. Badria, *Bioorg. Med. Chem.,* 2009, **17**, 5096.
29. N. F. Eweiss and A. Osman, *J. Heterocycl. Chem.,* 1980, **17**, 1713.
30. A. S. Shawali and A. O. Abdelhamid, *Bull. Chem. Soc. Jpn.,* 1976, **49**, 321.
31. R. N. Butler, *Chem. Rev*., 1975, **75**, 241.
32. P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. I. Warren, H. Bokesch, S. Kenney, and M. R. Boyd, *J. Nat. Cancer Inst.,* 1990, **82**, 1107.