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ARTICLE

Preparation of novel azidopyrazole derivatives with anticipated cytotoxic and antimicrobial activities

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Abstract

The reactions of azidopyrazole with some of primary amines and active methylene compounds were studied. Structures of the prepared compounds were identified using spectroscopic techniques. The reactions mechanisms were also proposed. The cytotoxic and antimicrobial activities were also examined for the newly synthesized compounds.

1 | INTRODUCTION

The pyrazole ring system is known to evoke wide range of biological activities.[1–9] The presence of pyrazole nucleus in many pharmacological agents has proved and leads to diverse applications in agriculture, technology, and medicine. Also, pyrazole ring system is one of azole family that described as anti-inflammatory, antifungal, antibacterial, anticancer, and antioxidant as well as antiviral agents.[10,11] In the same manner, aromatic amines are important reagents for the preparation of pharmaceuticals, pesticides, additives, dyes, and for structure-biological activity studies related to food carcinogens.[12–15] Moreover, azides are important building blocks due to their potential application in organic and bioorganic chemical reactions.[16–18] In the light of this survey and our interest in synthesis of heterocyclic chemistry,[1,19–23] we have now studied the reactivity of azidopyrazole carbaldehyde **1** towards certain active methylenes (**2a-e**, **3**), primary amines (**4a-g**), and hydrazines (**4h-k**) aiming at preparing new heterocycles of anticipated anticancer and antimicrobial activities (Figure 1).

2 | RESULTS AND DISCUSSION

Azidopyrazole carbaldehyde **1** was prepared by the reaction of chloropyrazole carbaldehyde with sodium azide in DMF at 0°C according to literature procedures.[24]

Condensation of **1** with the active methylene compounds **2a-d** proceeded in absolute ethanol at ambient temperature to yield the respective ethylenes **5a-d** (Scheme 1). The IR spectra of the prepared ethylenes indicated the presence of an azido functionality around 2140 cm^{-1} while the aldehydic C═O absorption around 1720 cm^{-1} was absent. In agreement with these findings, no signal for the aldehydic proton (δ_H 10.00 ppm) was detected in the ^1H NMR spectra.

Compound **1** was also reacted at boiling temperature with ethyl cyanoacetate **2e** in methylene chloride in under basic condition of NaOMe resulting in a mixture of pyrazol-2-cyanoacrylate derivative **6** in a 55% yield and pyrazolo[3,4-*d*][1,2,3]triazine derivatives (**7**) in a 20% yield (Scheme 1).

Elemental analyses, IR, and ^1H NMR measurements were compatible with the assigned structures. The IR spectrum of compound **6** indicated the presence of absorption bands at 2225 (CN), 2111 (N_3), and 1712 cm^{-1} (C═O). The IR spectrum of compound **7** confirmed the presence of bands at 3365 (NH), 2184 (CN), 1707 (CO, ester), and 1668 cm^{-1} (N═N).[15] The ^1H NMR spectrum of **6** revealed the presence of an exocyclic ethylenic proton at δ_H 7.81, which was absent in the spectrum of compound **7**.

Upon refluxing compound **1** with 2*H*-indene-1,-3-dione (**3**) in ethanol for 1 hour, 2-((5-azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methylene)-2*H*-indene-1,-3-dione (**8**) was solely obtained. It showed a band at 2226 cm^{-1} (N_3) in its IR spectrum. A signal at δ_H 8.01 ppm (CH═N) was detected in its ^1H NMR spectrum as well as two signals at δ_c 186.6, 188.8 (2 CO) in its ^{13}C NMR spectrum.

The reaction of compound **1** with the appropriate hydrazines **4a-c** was performed in ethanol at room temperature under stirring. In each case, a mixture of three products was obtained (*cf.* **9**, **10a-c**, and **11a-c**, Scheme 2).

The structures of all products were determined with elemental and spectroscopic analyses (Section 4). As distinguishing features, the IR spectra of **10a-c** and **11a-c** lacked the carbonyl amide absorption, which was detected at 1667 cm^{-1} in the spectrum of compound **9**.

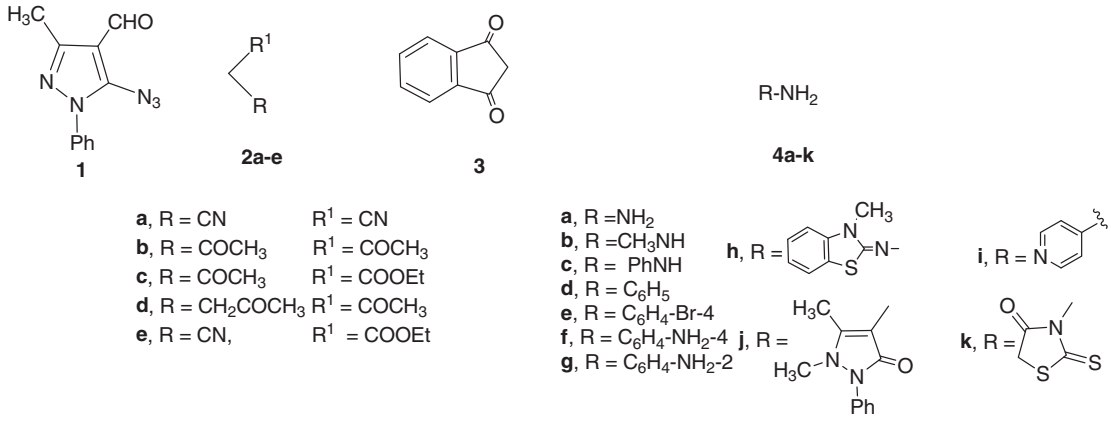
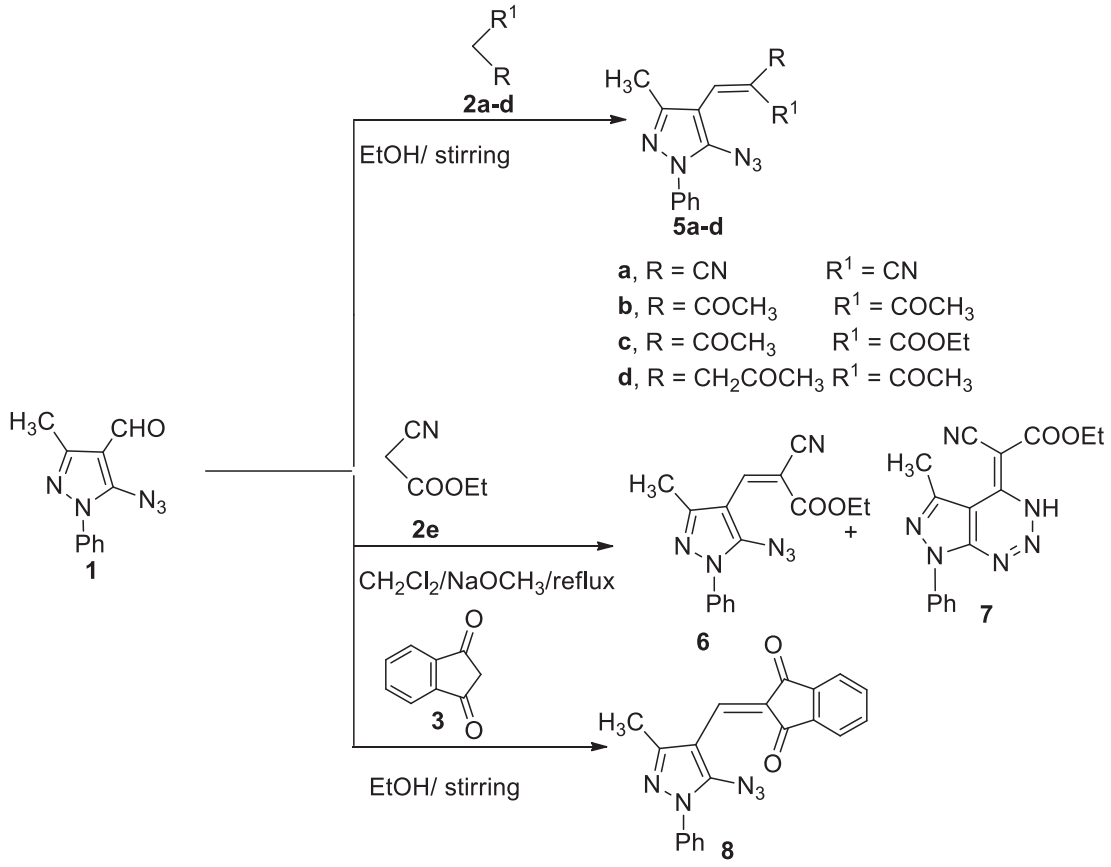
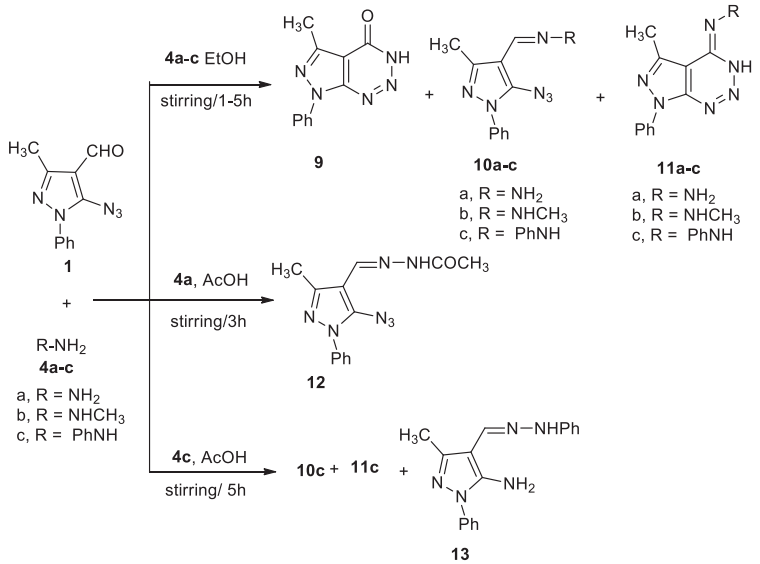


FIGURE 1 The reactants



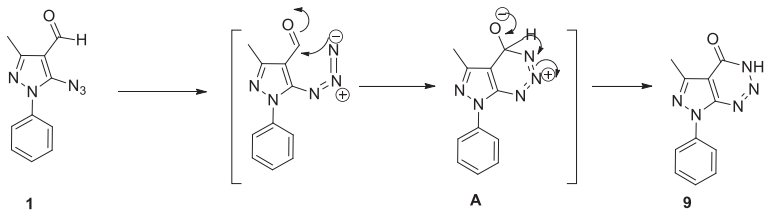
SCHEME 1 Reactions of azidopyrazole (**1**) with compounds that contain active methylene

SCHEME 2 Reactions of azidopyrazole (**1**) with hydrazines **4a-c**



On the other hand, unlike **10a-c**, compounds **9** and **11a-c** showed strong absorption band around 1660 cm^{-1} due to the N═N stretching vibration.[25]

The reaction of compound **1** with hydrazine hydrate (**4a**) was conducted in acetic acid at room temperature. A brown crystalline material was obtained and named as *N′*-((5-azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methylene)acetohydrazide (**12**). On the other hand, under the same experimental conditions, the reaction of **1** with phenyl hydrazine afforded a



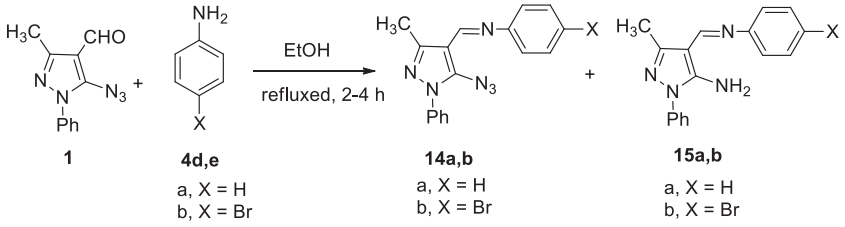
SCHEME 3 Formation mechanism[16,26] of compound **9**

mixture of substances including 3-methyl-1-phenyl-4-((2-phenylhydrazono)methyl)-1*H*-pyrazol-5-amine (**13**), **10c**, and **11c**.

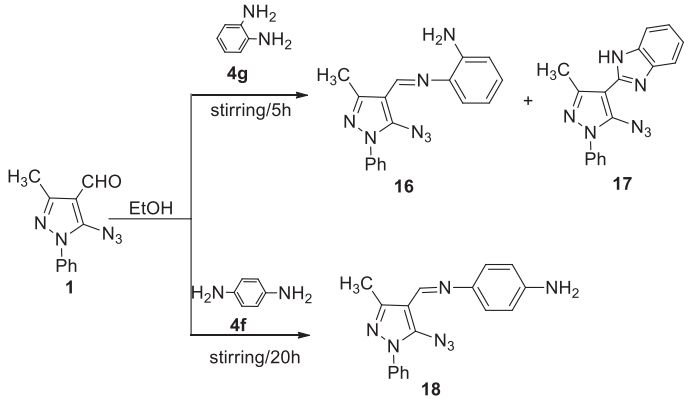
A possible explanation for the formation of compound **9** from the reaction of **1** with the hydrazine reagents **4a-c** is outlined in Scheme 3. Initial attack by the anion derived from the azido function on the most electron deficient center in the formyl group would afford the dipolar species "**A**," which undergoes intramolecular rearrangement to afford **9**. It has been reported that *o*-azido-carbaldehyde compounds can be intracyclized with good yield under the influence of a basic medium.[16,26]

The reaction of compound **1** with the primary amines **4d** and **4e** was also investigated. Compound **1** reacted with one molar equivalent of **4d** or **4e** in ethanol at under reflux yielding a mixture of the known Schiff bases **14a** or **14b**[27] and their α-amino pyrazoles **15a** or **15b** in each case (Scheme 4). The structural assignment of compounds **14a** or **14b** and **15a** or **15b** was based upon elemental analysis and spectroscopic data (cf. Section 4).

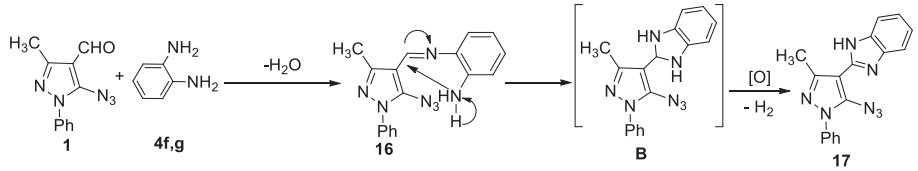
Condensation of **1** with *o*-phenylenediamine **4g** proceeded in ethanol at room temperature to give a mixture of *N*-1-((5-azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl) methylene)benzene-1,2-diamine (**16**) and 2-(5-azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1*H*-benzo[*d*]imidazole (**17**). On the other hand, under the same conditions, the reaction of **1** with *p*-phenylenediamine **4f** yielded *N*-1-((5-azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methylene)benzene-1,4-diamine (**18**) as the sole product (Scheme 5). Structures of compounds **16-18** were



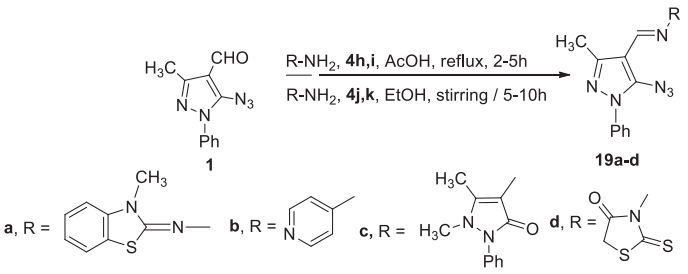
SCHEME 4 Reactions of azidopyrazole (**1**) with aniline derivatives **4d**,**e**



SCHEME 5 Reactions of azidopyrazole (**1**) with *o*- and *p*-phenyelendiamine



SCHEME 6 Formation mechanism of compound **17**



SCHEME 7 Reactions of azidopyrazole (**1**) with some heterocyclic amines (**4h-k**)

assigned on the basis of their IR, ^{1}H, ^{13}C NMR, and MS spectroscopic data (cf. Section 4).

Formation of **17** is proposed in terms of molecular rearrangement of the initially formed 1**6** to give a transient intermediate **B**, which readily undergoes dehydrogenation (auto-oxidation) yielding the final product **17**. This reaction is similar to the reactions of **3b** with carbonyl compounds[28] (Scheme 6).

When compound **1** was reacted with various heterocyclic amines **4h-k** in ethanol at room temperature, pure crystalline products (**19a-d**) were formed (Scheme 7). The structures of these compounds were determined based on their spectroscopic data. An absorption band was detected in the IR spectra of **19a-d** due to the azido functionality around 2100 cm^{-1}. No absorption band of the carbonyl group (C═O) was detected around 1700 cm^{-1}, which was supported by the absence of the signal of the aldehydic proton at δ_H 10 ppm in their ^{1}H NMR spectra (Scheme 7).

TABLE 1 Cytotoxicity of azide analogs against different cancer cell lines for 72 h

	Molt 4	U937	Sup-T1	562	LNcap
5a	NA	NA	NA	NA	NA
5b	10.45	18.47	8.41	10.56	NA
6	NA	4.46	13.1	NA	NA
9	NA	NA	NA	NA	NA
10a	NA	NA	NA	NA	NA
10b	NA	NA	19.07	NA	NA
11c	12.18	NA	NA	18.56	NA
12	25.09	NA	27.54	10.90	NA
15a	13.02	NA	11.27	14.20	NA
16	17.24	NA	12.43	28.49	NA
17	NA	NA	17.76	NA	NA
19a	NA	NA	NA	NA	NA
19b	NA	NA	NA	NA	NA
19c	NA	NA	NA	NA	NA
19d	13.55	NA	26.18	11.52	NA
Doxorubicin	0.001	0.001	0.006	0.08	0.96

Note. NA, not active at 20 μg/mL for 72-h evaluation.

3 | BIOLOGICAL ACTIVITY

3.1 | The cytotoxic activity

The cytotoxic activity of some of the prepared compounds was evaluated against Molt 4, U937, Sup-T1, 562, and LNcap cancer cell lines, and the results are depicted in Table 1. Among the tested compounds, 3-((5-azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methylene)pentane-2,4-dione (**5b**) exhibited potent activity against Molt 4, U937, Sup-T1, and 562 cells with IC_{50} 10.45, 18.47, 8.41, and 10.56 μg/mL, respectively after 72 hours. On the other hand, its analogue, which carries two cyano groups, 2-((5-azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl) methylene)malononitrile (**5a**), did not show any activity (Table 1). The derivative with one cyano and one ethyl acetate group, ethyl 3-(5-azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2-cyanoacrylate (**6**), showed activity against U937 and Sup-T1 cells with IC_{50} 4.46 and 13.1 μg/mL, respectively. The azido methyl hydrazine derivative showed activity against Sup-T1 cells with IC_{50} 19.07 μg/mL while the azido hydrazine derivative did not show any activity (Table 1). The triazine phenyl hydrazine derivative, 1-(5-methyl-7-phenyl-3*H*-pyrazolo[3,4-*d*][1,2,3]triazin-4(7*H*)-ylidene)-2-phenylhydrazine (**11c**), showed activity against Molt 4 and 562 cells with IC_{50} 12.18 and 18.56 μg/mL. The azido acetohydrazide derivative, *N*′-((5-azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl) methylene)acetohydrazide (**12**), exhibited activity against Molt4, Sup-T1, and 562 cells with IC_{50} 25.09, 27.54, and 10.90 μg/mL, respectively (Table 1). The phenyl pyrazol derivative, 3-methyl-1-phenyl-4-((phenylimino)methyl)-1*H*-pyrazol-5-amine (**15a**), also exhibited activity against Molt4, Sup-T1, and 562 cells with IC_{50} 13.02, 11.27, and 14.20 μg/mL, respectively. Moreover, the azido phenyl pyrazol derivative, *N*-1-((5-azido-3-methyl-1-phenyl-1*H*-

pyrazol-4-yl)methylene)benzene-1,2-diamine (**16**), was active against the same cancer cell lines Molt4, Sup-T1, and 562 cells with IC_{50} 17.24, 12.43, and 28.49 μg/mL, respectively (Table 1). A closely similar derivative with ortho amino group, *N*-1-((5-azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methylene)benzene-1,4-diamine (**18**), showed activity against a single cancel line, Sup-T1, with IC_{50} 17.76 μg/mL. The azido pyrazol thioxothiazolidin derivative, 3-((5-azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methyleneamino)-2-thioxothiazolidin-4-one (**19d**), demonstrated activity against Molt4, Sup-T1, and 562 cells with IC_{50} 13.55, 26.18, and 11.52 μg/mL, respectively (Table 1).[29,30]

3.2 | The antimicrobial activity

The antimicrobial activity of some of the prepared compounds was evaluated against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, and the results are depicted in Table 2. The tested compound showed no activity except for **17**, which showed moderate activity against *S. aureus* (Table 2). The lack of antimicrobial activity suggested that compounds, which were active against cancer cell lines, were only specific to those cell lines and did not kill any type of cells. The selectivity and safety profile of the tested compounds requires further studies.[31–33]

TABLE 2 MIC for 16 compounds (N = 3)

	MIC, μg/mL		
	Staphylococcus aureus	***Escherichia coli***	***Pseudomonas aeruginosa***
5a	NA	NA	NA
5b	128	NA	NA
6	NA	NA	NA
9	NA	NT	NT
10a	NA	NA	NA
10b	NA	NA	NT
11c	64	NA	NA
12	128	NA	NA
15a	NA	NA	NA
16	NA	NT	NT
17	32	NA	NA
18	NA	NA	NA
20a	NA	NT	NT
19b	NA	NT	NT
19c	NA	NA	NA
19d	64	NA	NA
Streptomycin[a]	NT	6.4	16
Penicillin G[a]	0.64	NT	NT

Abbreviations: MIC, minimum inhibitory concentrations; NA, no activity at 128 μg/mL; NT, no test.
[a]As positive control.

4 | EXPERIMENTAL

Melting points were determined in open glass capillaries using Electrothermal IA 9000 series digital melting point apparatus (Electrothermal, Essex, UK) and are uncorrected. The IR spectra were measured in KBr pellets with a Perkin-Elmer Infracord Spectrophotometer model 157(Grating). The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on JEOL-500 MHz Spectrometer, and the chemical shifts were recorded in δ values relative to TMS. The mass spectra were performed at 70 eV on a Shimada GCS-OP 1000 Ex Spectrometer provided with a data system. Elemental analyses were performed using Elmenter Varu EL Germany Instrument. The reported yields are based upon pure materials isolated by column chromatography. Solvents were dried/purified according to conventional procedures.

4.1 | Reaction of 5-azido-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (1) with the active methylene (2a-d)

4.1.1 | General procedure

In a 25-mL round bottom flak equipped with magnetic stirrer bar, a mixture of azidopyrazole (**1**, 0.1 mol) and 0.2 mol of malononitrile (**2a**), acetylacetone (**2b**), ethyl acetoacetate (**2c**), or acetonyl acetone (**2d**) in 10-mL absolute ethanol was stirred at room temperature for 1 to 48 hours (TLC monitoring). Few drops of pyridine were added to the reaction mixture of compound **1** with **2a**. The volatile materials were evaporated, the residual precipitate washed with petroleum ether, diethyl ether, dried, and recrystallized from ethanol.

*2-((5-Azido-3-methyl-1-phenyl-1H-pyrazol-4-yl) methylene)malononitrile (**5a**)*

Pale yellow crystals; yield 95%; m.p. 209°C to 211°C R_f = 0.64 (ethyl acetate/cyclohexane = 30/70); IR (KBr): $\tilde{\nu}$ = 2239 (br, 2CN), 2134 (N_3), 1612 (C=C), 1536 (C=N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.65 (s, 3 H, CH_3), 7.34 (s, 1 H, =C<u>H</u>), 7.52-8.32 (m, 5H, CH_{arom}) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.9 (CH_3), 73.7, 156.3

(C═C), 114.2, 116.6 (CN), 124.4, 128.2, 129.1, 131.2 (aromatic, C-H), 105.9, 138.9 (cyclic C═C), 150.2 (cyclic C═N) ppm. MS (EI, 70 eV): m/z (%) = 275 (10%) $[M]^+$, 247(15) $[M\text{-}28]^+$; Anal. for $C_{14}H_9N_7$ (275.27): calcd. C, 61.09; H, 3.30; N, 35.62; found: C, 61.12; H, 3.28; N, 35.65.

*3-((5-Azido-3-methyl-1-phenyl-1H-pyrazol-4-yl) methylene)pentane-2,4-dione (**5b**)*

Red solid; yield 87%; m.p. 156°C to 158°C R_f = 0.44 (ethyl acetate/cyclohexane = 30/70); IR (KBr): $\tilde{\nu}$ = 2147 (N_3), 1672, 1649 (C═O), 1592 (C═C), 1527 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.27, 2.28 (2 s, 6 H, $2CH_3$), 2.63 (s, 3 H, CH_3), 7.45-8.29 (m, 5 H, CH_{arom}), 8.32 (s, 1 H, CH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.4 (CH_3), 29.4 ($COCH_3$), 104.1, 138.7 (cyclic C═C), 124.7, 128.2, 129.7, 134.1 (aromatic, C─H), 148.2, 148.4 (C═C), 150.5 (cyclic C═N), 201.8, 203.5 (C═O) ppm. MS (EI, 70 eV): m/z (%) = 309 (5%) $[M]^+$; Anal. for $C_{16}H_{15}N_5O_2$ (309.32): calcd. C, 62.13; H, 4.89; N, 22.64; found: C, 62.16; H, 4.95; N, 22.53.

*Ethyl 2-((5-azido-3-methyl-1-phenyl-1H-pyrazol-4-yl) methylene)-3-oxobutanoate (**5c**)*

Brown crystals, yield 80%; m.p. 165°C to 167°C R_f = 0.62 (ethyl acetate/cyclohexane = 30/70); IR (KBr): $\tilde{\nu}$ = 2124 (N_3), 1674, 1629 (C═O), 1598 (C═C), 1527 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 1.36 (t, 3 H, CH_3), 2.15 (s, 3 H, CH_3), 2.45 (s, 3 H, CH_3), 4.90 (q, 2 H, CH_2), 7.45-7.69 (m, 5 H, CH_{arom}), 9.17 (s, 1 H, CH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.9 (CH_3), 15.9 (CH_3), 29.4 ($COCH_3$), 58.8 (CH_2), 106.1, 131.2 (cyclic C═C), 96.4, 138.3 (C═C), 150.7 (cyclic C═N), 125.5, 127.1, 128.2, 129.3, 131.1 (aromatic, C─H), 168.7 (C═O), 198.2 (C═O) ppm. MS (EI, 70 eV): m/z (%) = 339 (3%) $[M]^+$; Anal. for $C_{17}H_{17}N_5O_3$ (339.35): calcd. C, 60.17; H, 5.05; N, 20.64; found: C, 60.12; H, 5.10; N, 20.62.

*3-((5-Azido-3-methyl-1-phenyl-1H-pyrazol-4-yl) methylene)hexane-2,5-dione (**5d**)*

Brown crystals; yield 20%; m.p. 172°C to 173°C R_f = 0.58 (ethyl acetate/cyclohexane = 30/70); IR (KBr): $\tilde{\nu}$ = 2215 (N_3), 1652 (C═O), 1595 (C═C), 1563 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.61 (s, 3 H, CH_3), 3.32 (s, 3 H, CH_3), 4.35 (s, 3 H, CH_3), 5.13 (s, 2 H, CH_2), 6.96 (s, 1 H, CH), 7.17-7.20 (m, 5 H, CH_{arom}) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.6 (CH_3), 23.0, 29.4 ($CO\mathit{CH_3}$), 53.4 (CH_2), 88.8, 142.6 (C═C), 104.1, 138.6 (cyclic C═C), 150.3 (cyclic C═N), 124.7, 127.1, 128.0, 131.5, 135.6, (aromatic, C─H), 181.9, 187.4 (C═O) ppm. MS (EI, 70 eV): m/z (%) = 323 (2%) $[M]^+$, 281 (2%) $[M\text{-}42]^+$; Anal. for $C_{17}H_{17}N_5O_2$ (323.35): calcd. C, 63.15; H, 5.30; N, 21.66; found: C, 63.21; H, 5.25; N, 21.68.

4.2 | Reaction of 5-azido-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (1) and ethyl 2-cyanoacetate (2e)

A mixture of compound **1**, (0.1 mol) and 0.1 mol of ethyl 2-cyanoacetate (**2e**), in 20-mL DCM in the presence of 0.1 g NaOMe was stirred in ice bath for 2 hours then refluxed for 2 hours (TLC monitoring, R_f = 0.31 (ethyl acetate/petroleum ether (60:80°C) = 30/70)). The reaction mixture was evaporated under reduced pressure, and the residual material was chromatographed on silica gel column to give products **6** and **7**.

4.2.1 | Ethyl 3-(5-azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2-cyanoacrylate (6)

Eluent: petroleum ether (60:80°C) /ethyl acetate (95/5, *v*/v). Product **6** was separated as red crystals, yield 55%; m.p. 120°C to 122°C. IR (KBr): $\tilde{\nu}$ = 2225 (CN), 2211 (N_3), 1712 (C═O), 1619, 1596 (C═C), 1550 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 1.40 (t, 3 H, CH_3), 2.67 (s, 3 H, CH_3), 4.41 (q, 2 H, CH_2), 7.30-7.71 (m, 5 H, CH_{arom}), 7.81 (s, 1 H, CH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.4 (CH_3), 15.7 (CH_3), 61.9 (CH_2), 117.1 (CN), 99.3, 155.1 (C═C), 105.9, 133.7 (cyclic C═C), 124.2, 127.1, 128.2, 129.1, 133.7 (aromatic, C-H), 150.3 (cyclic C═N), 164.9 (C═O) ppm. MS (EI, 70 eV): m/z (%) = 322 (20%) $[M]^+$; Anal. for $C_{16}H_{14}N_6O_2$ (322.32): calcd. C, 59.62; H, 4.38; N, 26.07; found: C, 59.88; H, 4.22; N, 25.99.

4.2.2 | Ethyl 2-cyano-2-(5-methyl-7-phenyl-3*H*-pyrazolo[3,4-*d*][1,2,3]triazin-4(7H)-ylidene)acetate (7)

Eluent: petroleum ether (60:80°C)/ethyl acetate (80/20, *v*/v). Product **7** was separated as yellow crystals, yield 20%; m.p. 150°C to 152°C. IR (KBr): $\tilde{\nu}$ = 3365 (NH), 2184 (CN), 1707 (C═O), 1668 (N═N), 1604 (C═C), 1585 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 1.31 (t, 3 H, CH_3), 3.80 (s, 3 H, CH_3), 4.28 (q, 2 H, CH_2), 6.84-7.53 (m, 5 H, CH_{arom}), 7.55 (s, 1H, NH, exchangeable with D_2O) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.9 (CH_3), 15.1 (CH_3), 63.2 (CH_2), 74.4 (═C─CN), 114.1 (CN), 95.5, 154.5 (cyclic C═C), 124.4, 124.7, 128.2, 129.3, 138.3 (aromatic, C─H), 146.3 (cyclic C═N), 169.4 (C═O) ppm. MS (EI, 70 eV): m/z (%) = 322 (30%) $[M]^+$; Anal. for $C_{16}H_{14}N_6O_2$ (322.32): calcd. C, 59.62; H, 4.38; N, 26.07; found: C, 59.78; H, 4.24; N, 26.02.

4.3 | Synthesis of 2-((5-azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methylene)-2*H*-indene-1,3-dione (8)

A mixture of compound **1** (1 mmol) and 1*H*-indene-1,3(2*H*)-dione (**3**, 1 mmol) in absolute ethanol (20 mL) was stirred at room temperature for 1 hour. The reaction mixture was evaporated under reduced pressure, and the residual material was chromatographed on silica gel column using petroleum ether (60:80°C)/ethyl acetate (95/5, *v*/v) as an eluent, to give **8** as colorless crystals; yield 70%; m.p. 150°C to 152°C R_f = 0.31 (ethyl acetate/petroleum ether (60:80°C) = 20/80); IR (KBr): $\tilde{\nu}$ = 2226 (N_3), 1626 (C═O), 1620 (C═O),1560 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.59 (s, 1 H, CH_3), 7.21-7.67 (m, 9 H, CH_{arom}), 8.01 (s, 1H, CH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.9 (CH_3), 124.4, 124.7, 127.1, 128.2, 129.1, 129.2, 133.7, 133.9 (aromatic, C—H), 104.1, 133.9 (cyclic C═C), 117.3, 143.8 (C═CH), 150.7 (cyclic C═N), 186.6, 188.8 (C═O) ppm. MS (EI, 70 eV): *m*/*z* (%) = 355 (2%) $[M]^+$; Anal. for $C_{20}H_{13}N_5O_2$ (355.35): calcd. C, 67.60; H, 3.69; N, 19.71; found: C, 67.56; H, 3.73; N, 19.67.

4.4 | Reaction of 1 and hydrazines (4a-c)

4.4.1 | General procedure

A mixture of compound **1** (5 mmol) and the appropriate hydrazines, namely, hydrazine hydrate, methyl hydrazine, and/or phenyl hydrazine (10 mmol) in absolute ethanol (25 mL) was stirred for 1 to 5 hours (TLC monitoring, R_f = 0.23 (ethyl acetate/cyclohexane = 30/70)). The reaction mixture was evaporated under reduced pressure, and the residual material was chromatographed on silica gel column to give products **9**, **10a-c**, and **11a-c**.

*5-Methyl-7-phenyl-3H-pyrazolo[3,4-d][1,2,3]triazin-4(7H)-one (**9**)*

Eluent: petroleum ether (60:80°C)/ethyl acetate (95/5, *v*/v). Product **9** was separated as orange crystals, yield 5%; m.p. 160°C to 162°C. IR (KBr): $\tilde{\nu}$ = 3326 (NH), 1672 (C═O), 1665 (N═N), 1619 (C═C), 1596 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.57 (s, 3 H, CH_3), 4.51 (s, 1 H, NH, exchangeable with D_2O), 7.49-7.56 (m, 5 H, CH_{arom}) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.5 (CH_3), 96.1, 138.7 (cyclic C═C), 124.4, 124.7, 128.2, 129.3, 129.9 (aromatic, C-H), 148.8 (C═N), 162.0 (C═O) ppm. MS (EI, 70 eV): *m*/*z* (%) = 227 (50%) $[M]^+$; Anal. for $C_{11}H_9N_5O$ (227.22): calcd. C, 58.14; H, 3.99; N, 30.82; found: C, 58.12; H, 4.01; N, 30.74.

*2-((5-Azido-3-methyl-1-phenyl-1H-pyrazol-4-yl) methylene)hydrazine (**10a**)*

Eluent: petroleum ether (60:80°C)/ethyl acetate (80/20, *v*/v). Product **10a** was separated as colorless crystals, yield 45%; m.p. 217°C to 219°C. IR (KBr): $\tilde{\nu}$ = 3410, 3277 (NH_2), 2360 (N_3), 1625 (C═N), 1594, 1574 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.59 (s, 3 H, CH_3), 7.31-7.75 (m, 5 H, CH_{arom}), 8.36 (s, 2 H, NH_2), 8.69 (s, 1 H, CH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.9 (CH_3), 105.7, 134.1 (cyclic C═C), 125.8, 127.1, 128.2, 129.3, 131.2 (aromatic, C—H), 138.1, 151.7 (2 C═N) ppm. MS (EI, 70 eV): *m*/*z* (%) = 241 (5%) $[M]^+$; Anal. for $C_{11}H_{11}N_7$ (241.25): calcd. C, 54.76; H, 4.60; N, 40.64; found: C, 54.68; H, 4.55; N, 40.59.

*2-((5-Azido-3-methyl-1-phenyl-1H-pyrazol-4-yl) methylene)-1-methylhydrazine (**10b**)*

Eluent: petroleum ether (60:80°C)/ethyl acetate (80/20, *v*/v). Product **10b** was separated as yellow crystals, yield 45%; m.p. 240°C to 242°C. IR (KBr): $\tilde{\nu}$ = 3333 (NH), 2218 (N_3), 1653 (C═C), 1595, 1564 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.36 (s, 3 H, CH_3), 4.51 (s, 3 H, CH_3), 6.85 (s, 1H, NH, exchangeable with D_2O), 7.26-7.54 (m, 5 H, CH_{arom}), 7.57 (s, 1 H, CH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.9 (CH_3), 35.7 (CH_3), 107.4, 134.3 (C═C), 124.5, 127.1, 128.2, 129.3, 138.1 (aromatic, C—H), 141.7, 151.4 (C═N) ppm. MS (EI, 70 eV): *m*/*z* (%) = 255 (5%) $[M]^+$, 198 (100%) $[M-58]^+$; Anal. for $C_{12}H_{13}N_7$ (255.28): calcd. C, 56.46; H, 5.13; N, 38.41; found: C, 56.50; H, 5.20; N, 38.39.

*2-((5-Azido-3-methyl-1-phenyl-1H-pyrazol-4-yl) methylene)-1-phenylhydrazine (**10c**)*

Eluent: petroleum ether (60:80°C)/ethyl acetate (60/40, *v*/v). Product **10c** was separated as orange crystals, yield 35%; m.p. 232°C to 234°C. IR (KBr): $\tilde{\nu}$ = 3291 (NH), 2211 (N_3), 1596 (C═C), 1553, 1543 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.45 (s, 3 H, CH_3), 4.80 (s, 1H, NH, exchangeable with D_2O), 5.95 (s, 1 H, CH), 6.86-7.65 (m, 10 H, CH_{arom}) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.8 (CH_3), 107.4, 133.7 (C═C), 115.8, 116.9, 124.7, 125.8, 127.1, 128.2,129.3, 142.2 (aromatic, C—H), 144.8, 151.4 (C═N) ppm. MS (EI, 70 eV): *m*/*z* (%) = 318 (5%) $[M + 1]^+$; Anal. for $C_{17}H_{15}N_7$ (317.35): calcd. C, 64.34; H, 4.76; N, 30.90; found: C, 64.43; H, 4.55; N, 30.85.

*1-(5-Methyl-7-phenyl-3H-pyrazolo[3,4-d][1,2,3]triazin-4(7H)-ylidene)hydrazine (**11a**)*

Eluent: petroleum ether (60:80°C)/ethyl acetate (65/35, *v*/v). Product **11a** was separated as orange crystals, yield 30%; m.p. 150°C to 152°C. IR (KBr): $\tilde{\nu}$ = 3432 (NH_2), 3346 (NH), 1680 (N═N), 1630 (C═C), 1596 (C═N), 1540 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.00 (s,

1 H, NH, exchangeable with D_2O), 2.36 (s, 3 H, CH_3), 4.51 (s, 2H, NH_2, exchangeable with D_2O), 7.26-7.57 (m, 5 H, CH_{arom}) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.5 (CH_3), 99.3, 156.2 (cyclic C═C), 124.4, 127.0, 128.2, 129.3, 138.7 (aromatic, C─H), 136.0, 145.6 (2 C═N) ppm. MS (EI, 70 eV): m/z (%) = 322 (20%) $[M]^+$; Anal. for $C_{11}H_{11}N_7$ (241.25): calcd. C, 54.76; H, 4.60; N, 40.64; found: C, 54.75; H, 4.64; N, 40.63.

*2-Methyl-1-(5-methyl-7-phenyl-3H-pyrazolo[3,4-d][1,2,3]triazin-4(7H)-ylidene)hydrazine (**11b**)*

Eluent: petroleum ether (60:80°C)/ethyl acetate (50/50, *v*/v). Product **11b** was separated as yellow crystals, yield 55%; m.p. 205°C to 207°C. IR (KBr): $\tilde{\nu}$ = 3380, 3226 (NH), 1668 (N═N), 1619 (C═C), 1551, 1530 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 1.27 (s, 1H, NH, exchangeable with D_2O), 2.39 (s, 3 H, CH_3), 2.58, 2.61 (d, 3 H, CH_3), 6.90 (s, 1H, NH), 7.46-7.80 (m, 5 H, CH_{arom}) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.9 (CH_3), 36.1 (CH_3), 100.0, 156.2 (cyclic C═C), 124.4, 127.1, 128.2, 129.3, 132.5, 138.7 (aromatic, C─H), 145.3 (C═N) ppm. MS (EI, 70 eV): m/z (%) = 257 (40%) $[M]^{+2}$, 227 (10%) [M-30]; Anal. for $C_{12}H_{13}N_7$ (255.28): calcd. C, 56.46; H, 5.13; N, 38.41; found: C, 56.49; H, 4.99; N, 38.29.

*1-(5-Methyl-7-phenyl-3H-pyrazolo[3,4-d][1,2,3]triazin-4(7H)-ylidene)-2-phenylhydrazine (**11c**)*

Eluent: petroleum ether (60:80°C)/ethyl acetate (60/40, *v*/v). Product **11c** was separated as orange crystals, yield 45%; m.p. 115°C to 117°C. IR (KBr): $\tilde{\nu}$ = 3414, 3307 (NH), 1689 (N═N), 1612 (C═C), 1597, 1542 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.36 (s, 3 H, CH_3), 5.30 (s, 1H, NH, exchangeable with D_2O), 7.13-7.69 (m, 10 H, CH_{arom}) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.2 (CH_3), 100.0, 143.8 (cyclic C═C), 115.3, 115.6, 123.8, 127.1, 128.2, 129.3, 138.1 (aromatic, C-H), 145.3, 150.3 (C═N) ppm. MS (EI, 70 eV): m/z (%) = 317 (10%) $[M]^+$; Anal. for $C_{17}H_{15}N_7$ (317.35): calcd. C, 64.34; H, 4.76; N, 30.90; found: C, 64.32; H, 4.74; N, 30.89.

4.5 | Synthesis of compounds 12 and 13

A mixture of compound **1** (5 mmol) hydrazine hydrate and/or phenyl hydrazine in acetic acid (25 mL) was stirred for 3 to 5 hours (TLC monitoring, R_f = 0.51 (ethyl acetate/cyclohexane = 30/70)). The reaction mixture was poured on ice cold water to give brown precipitate, which was filtered, washed with cold water, and recrystallized from acetone to give product **12**, and/or the residual material was chromatographed on silica gel column to give compounds **10c**, **11c**, and **13** in case of reacting compound **1** with phenyl hydrazine. Compounds **10c** (40% yield) and **11c** (25% yield) were isolated and identified by comparing their melting points and IR spectra with those of the previously isolated specimens.

4.5.1 | *N′*-((5-Azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methylene)acetohydrazide (12)

Brown crystals, 85% yield (acetone); m.p. 140°C to 142°C R_f = 0.42 (ethyl acetate/petroleum ether (60:80°C) = 30/70); IR (KBr): $\tilde{\nu}$ = 3046 (NH), 2146 (N_3), 1628 (C═O), 1591 (C═C), 1541, 1503 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.39 (s, 3 H, CH_3), 2.60 (s, 1H, NH, exchangeable with D_2O), 7.46-7.80 (m, 5 H, CH_{arom}), 8.50 (s, 1H, CH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.9 (CH_3), 22.9 ($CO\mathit{CH_3}$), 107.0, 133.7 (cyclic C═C), 125.8, 127.1, 128.2, 129.3, 138.3 (aromatic, C─H), 150.7, 151.1 (C═N) ppm. MS (EI, 70 eV): m/z (%) = 283 (5%) $[M]^+$, 183 (40%)[M-100]; Anal. for $C_{13}H_{13}N_7O$ (283.29): calcd. C, 55.12; H, 4.63; N, 34.61; found: C, 55.18; H, 4.53; N, 34.45.

4.5.2 | 3-Methyl-1-phenyl-4-((2-phenylhydrazono)methyl)-1*H*-pyrazol-5-amine (13)

Eluent: petroleum ether (60:80°C)/ethyl acetate (50/50, *v*/v). Product **13** was separated as yellow crystals, yield 25%; m.p. 156°C to 158°C. IR (KBr): $\tilde{\nu}$ = 3446 (NH_2), 3322 (NH), 1612 (C═C), 1598, 1583 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.29 (s, 3 H, CH_3), 2.70 (s, 1H, NH, exchangeable with D_2O), 7.20 (s, 2H, NH_2, exchangeable with D_2O), 7.28-7.80 (m, 10 H, CH_{arom}), 8.85 (s, 1H, CH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.8 (CH_3), 98.0, 142.2 (cyclic C═C), 115.7, 125.8, 127.1, 128.2, 129.1, 129.3, 138.7, 144.4 (aromatic, C─H), 144.8, 153.6 (C═N) ppm. MS (EI, 70 eV): m/z (%) = 291 (5%) $[M]^+$; Anal. for $C_{17}H_{17}N_5$ (291.35): calcd. C, 70.08; H, 5.88; N, 24.04; found: C, 70.10; H, 5.90; N, 24.02.

4.6 | Reaction of 1 with primary amines (4d,e)

A mixture of compound **1** (5 mmol) and the appropriate amine, namely, aniline, and/or *p*-bromo aniline (10 mmol) in absolute ethanol (25 mL) was refluxed for 2 to 4 hours (TLC monitoring, R_f = 0.44 (ethyl acetate/petroleum ether (60:80°C) = 50/50)). The reaction mixture was evaporated under reduced pressure, and the

residual material was chromatographed on silica gel column to give products **14a**,**b** and **15a**,**b**.

4.6.1 | *N*-((5-Azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methylene)benzenamine (14a)

Eluent: petroleum ether (60:80°C)/ethyl acetate (93/7, *v*/v). Product **14a** was separated as yellow crystals, yield 15%; m.p. 68-70 (60:80°C) C (lit. 68°C)[15]. IR (KBr): $\tilde{\nu}$ = 2225 (N_3), 1551, 1503 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.50 (s, 3 H, CH_3), 7.50-8.01 (m, 10 H, CH_{arom}), 8.60 (s, 1 H, CH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.9 (CH_3), 110.5, 138.7 (C═C), 121.9, 122.6, 124.7, 125.5, 128.2, 129.3, 129.9, 130.4, 140.2 (aromatic, C─H), 151.4, 153.8 (C═N) ppm. MS (EI, 70 eV): *m*/*z* (%) = 304 (15%) $[M + 2]^+$, 274 (35); Anal. for $C_{17}H_{14}N_6$ (302.33): calcd.: C, 67.54; H, 4.67; N, 27.80; found: C, 67.45; H, 4.72; N, 27.70.

4.6.2 | *N*-((5-Azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methylene)-4-bromobenzenamine (14b)

Eluent: petroleum ether (60:80°C)/ethyl acetate (90/10, *v*/v). Product **14b** was separated as yellow crystals, yield 10%; m.p. 163-165 (60:80°C) C. IR (KBr): $\tilde{\nu}$ = 2214 (N_3), 1595, 1539 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.49 (s, 3 H, CH_3), 7.22-8.04 (m, 9 H, CH_{arom}), 9.13 (s, 1 H, CH)ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.9 (CH_3), 108.3, 140.7 (C═C), 122.6, 122.7, 123.9, 124.2, 128.2, 129.3, 129.6, 131.2, 138.3 (aromatic, C─H), 151.4, 154.5 (C═N) ppm. MS (EI, 70 eV): *m*/*z* (%) = 381 (10%), 382 (8%), 383 (4%) $[M]^+$; Anal. for $C_{17}H_{13}BrN_6$ (381.23): calcd.: C, 53.56; H, 3.44; Br, 20.96; N, 22.04; found: C, 53.55; H, 3.43; Br, 20.99; N, 22.14.

4.6.3 | 3-Methyl-1-phenyl-4-((phenylimino)methyl)-1*H*-pyrazol-5-amine (15a)

Eluent: petroleum ether (60:80°C)/acetone (80/20, *v*/v). Product **15a** was separated as orange crystals, yield 75%; m.p. 148°C to 150°C. IR (KBr): $\tilde{\nu}$ = 3290 (NH_2), 2211 (N_3), 1596, 1543 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.39 (s, 3 H, CH_3), 2.80 (s, 2H, NH_2, exchangeable with D_2O), 6.10 (s, 1H, CH), 6.92-7.75 (m, 10 H, CH_{arom}) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.9 (CH_3), 100.0, 151.6 (cyclic C═C), 121.2, 124.7, 125.2, 125.8, 127.9, 128.2, 129.3, 129.9, 130.3, 138.5 (aromatic, C-H), 142.2, 156.3 (C═N) ppm. MS (EI, 70 eV): *m*/*z* (%) = 274 (100%) $[M]^{-2}$; Anal. for $C_{17}H_{16}N_4$ (276.34): calcd. C, 73.89; H, 5.84; N, 20.27; found: C, 73.90; H, 5.89; N, 20.30.

4.6.4 | 4-((4-Bromophenylimino)methyl)-3-methyl-1-phenyl-1*H*-pyrazol-5-amine (15b)

Eluent: petroleum ether (60:80°C)/acetone (90/10, *v*/v). Product **15b** was separated as golden crystals, yield 85%; m.p. 177°C to 179°C. IR (KBr): $\tilde{\nu}$ = 3317 (NH_2), 2192 (N_3), 1596, 1540 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 1.62 (s, 2H, NH_2, exchangeable with D_2O), 2.39 (s, 3 H, CH_3), 5.94 (s, 1H, CH), 6.81-7.46 (m, 9H, CH_{arom}) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.1 (CH_3), 98.7, 151.9 (cyclic C═C), 119.5, 120.0, 123.8, 124.4, 125.8, 128.2, 129.3, 132.0, 138.6, 142.2 (aromatic, C─H), 154.4, 155.9 (C═N) ppm. MS (EI, 70 eV): *m*/*z* (%) = 354 (2%), 356 (3%) $[M]^{+2}$; Anal. for $C_{17}H_{15}BrN_4$ (355.23): calcd. C, 57.48; H, 4.26; Br, 22.49; N, 15.77; found: C, 57.50; H, 4.25; Br, 22.50; N, 15.73.

4.7 | Reaction of 5-azido-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (1) with *p*- and *o*-phenylenediamine (4g,f)

4.7.1 | General procedure

o- or *p*-Phenylenediamine (**4g** or **4f**) (0.66 g, 5 mmol) was added to a stirred solution of compound **1** (2.27 g, 10 mmol) in 20-mL ethanol. The mixture was stirred for 5 to 7 hours (TLC-controlled, R_f = 0.44 (ethyl acetate/petroleum ether (60:80°C) = 50/50)). The reaction mixture was evaporated under reduced pressure, and the residual material was chromatographed on silica gel column to give products **16** and **17** when we used **4g** and give **18** when we used **4f**.

*N-1-((5-Azido-3-methyl-1-phenyl-1H-pyrazol-4-yl) methylene)benzene-1,2-diamine (**16**)*

Eluent: petroleum ether (60:80°C)/ethyl acetate (95/5, *v*/v). Product **16** was separated as orange crystals, yield 5%; m.p. 217°C to 219°C. IR (KBr): $\tilde{\nu}$ = 3440 (NH_2), 2225 (N_3), 1597, 1551 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 1.64 (s, 2H, NH_2, exchangeable with D_2O), 2.75 (s, 3 H, CH_3), 7.28-7.59 (m, 9 H, CH_{arom}), 9.60 (s, 1 H, CH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.5

(CH_3), 108.5, 142.4 (C═C), 117.1, 124.7, 125.8, 127.1, 128.2, 129.3, 129.7, 131.9, 139.8, 145.4 (aromatic, C-H), 151.4, 156.2 (C═N) ppm. MS (EI, 70 eV): m/z (%) = 315 (15%) $[M\text{-}2H]^+$, Anal. for $C_{17}H_{15}N_7$ (317.35): calcd. C, 64.34; H, 4.76; N, 30.90; found: C, 64.36; H, 4.75; N, 30.86.

*2-(5-Azido-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1H-benzo[d]imidazole (**17**)*

Eluent: petroleum ether (60:80°C)/ethyl acetate (93/7, *v*/v). Product **17** was separated as colorless crystals, yield 85%; m.p. 235°C to 237°C. IR (KBr): $\tilde{\nu}$ = 3272 (NH), 2237 (N_3), 1594, 1543 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.90 (s, 3 H, CH_3), 7.22-8.04 (m, 9 H, CH_{arom}), 9.94 (s, 1H, NH, exchangeable with D_2O) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.5 (CH_3), 90.1, 146.3 (C═C), 115.4, 118.5, 123.1, 124.4, 126.2, 128.9, 137.4, 139.6, 151.4 (aromatic, C-H), 166.1 (C═N) ppm. MS (EI, 70 eV): m/z (%) = 315 (2%) $[M]^+$, Anal. for $C_{17}H_{13}N_7$ (315.33): calcd. C, 64.75; H, 4.16; N, 31.09; found: C, 64.65; H, 4.20; N, 31.10.

*N-1-((5-Azido-3-methyl-1-phenyl-1H-pyrazol-4-yl) methylene)benzene-1,4-diamine (**18**)*

Eluent: petroleum ether (60:80°C)/ethyl acetate (90/10, *v*/v). Product **18** was separated as deep red crystals, yield 88%; m.p. 173°C to 175°C. IR (KBr): $\tilde{\nu}$ = 3433 (NH_2), 2141 (N_3), 1616 (C═C), 1590, 1545 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.47 (s, 3 H, CH_3), 6.20 (s, 2H, NH_2, exchangeable with D_2O), 6.63-8.04 (m, 10 H, CH_{arom}), 8.78 (s, 1 H, CH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.8 (CH_3), 109.3, 138.6 (C═C), 118.7, 119.7, 123.1, 124.4, 125.8, 128.2, 129.3, 140.7 (aromatic, C-H), 148.9, 156.2 (C═N) ppm. MS (EI, 70 eV): m/z (%) = 317 (2%) $[M]^+$, Anal. for $C_{17}H_{15}N_7$ (317.35): calcd. C, 64.34; H, 4.76; N, 30.90; found: C, 64.35; H, 4.73; N, 30.95.

4.8 | Reaction of 1 with heterocyclic amines (4h-k)

4.8.1 | General procedure

Benzothiazole hydrazine **4h** and/or 4-amino pyridine **4i** (5 mmol) was added to a stirred solution of compound **1** (2.27 g, 10 mmol) in 20-mL glacial acetic acid. The mixture was refluxed for 2 to 5 hours (TLC controlled, R_f = 0.40 (ethyl acetate/petroleum ether (60:80°C) = 50/50)). The reaction mixture after cooling was poured on ice water, the precipitate formed was collected by filtration and recrystallized from ethanol to give compound **19a,b**.

*1-((5-Azido-3-methyl-1-phenyl-1H-pyrazol-4-yl) methylene)-2-(3-methylbenzo[d]thiazol-2(3H)-ylidene) hydrazine (**19a**)*

Product **19a** was separated as yellow crystals, yield 65%; m.p. 210°C to 212°C. IR (KBr): $\tilde{\nu}$ = 2225 (N_3), 1616 (C═C), 1572, 1545 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.34 (s, 3 H, CH_3), 3.69 (s, 3 H, CH_3), 7.12-8.49 (m, 9 H, CH_{arom}), 8.52 (s, 1 H, CH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.2 (CH_3), 29.4 (CH_3), 109.0, 139.5 (C═C), 112.6, 122.7, 123.1, 124.3, 124.5, 127.1, 128.2, 129.3, 132.5 138.3 (aromatic, C─H), 151.4, 152.5, 166.6 (C═N) ppm. MS (EI, 70 eV): m/z (%) = 388 (2%) $[M]^+$, Anal. for $C_{19}H_{16}N_8S$ (388.45): calcd. C, 58.75; H, 4.15; N, 28.85; S, 8.25; found: C, 58.77; H, 4.13; N, 28.90; S, 8.20.

*N-((5-Azido-3-methyl-1-phenyl-1H-pyrazol-4-yl) methylene)pyridin-4-amine (**19b**)*

Product **19b** was separated as yellow crystals, yield 82%; m.p. 169°C to 171°C. IR (KBr): $\tilde{\nu}$ = 2226 (N_3), 1615 (C═C), 1628, 1598, 1562 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.36 (s, 3 H, CH_3), 7.40-8.42 (m, 9 H, CH_{arom}), 9.13 (s, 1 H, CH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.3 (CH_3), 105.4, 142.4 (C═C), 114.7, 122.9, 123.9, 126.8, 129.4, 137.1, 138.8, 149.2 (aromatic, C-H), 149.9, 161.4 (C═N) ppm. MS (EI, 70 eV): m/z (%) = 303 (10%) $[M]^+$; Anal. for $C_{16}H_{13}N_7$ (303.32): calcd. C, 63.36; H, 4.32; N, 32.32; found: C, 63.38; H, 4.23; N, 32.35.

4.9 | Reaction of 1 with heterocyclic amines (4j,k)

4.9.1 | General procedure

Antipyrine **4j** or aminorhodanine **4k** (5 mmol) was added to a stirred solution of compound **1** (2.27 g, 10 mmol) in 20-mL ethanol. The mixture was stirred for 5 to 7 hours (TLC controlled, R_f = 0.22 (ethyl acetate/petroleum ether (60:80°C) = 50/50)). The precipitate formed was collected by filtration and recrystallized from ethanol to give compounds **20c** or **20d**.

*4-((5-Azido-3-methyl-1-phenyl-1H-pyrazol-4-yl) methyleneamino)-1,2-dihydro-1,5-dimethyl-2-phenylpyrazol-3-one (**19c**)*

Product **19c** was separated as yellow crystals, yield 66%; m.p. 156°C to 158°C. IR (KBr): $\tilde{\nu}$ = 2207 (N_3), 1593, 1548 (C═N) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.39 (s, 3 H, CH_3), 2.60 (s, 3 H, CH_3), 3.28 (s, 3 H, CH_3), 7.21-7.67 (m, 10 H, CH_{arom}), 8.00 (s, 1 H, CH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.1, 14.3, 39.5 (CH_3), 105.4,

142.4, 110.1, 150.2 (C═C), 122.9, 123.9, 126.8, 129.4, 133.1, 139.8 (aromatic, C─H), 149.9 (C═N), 160.8 (C═O), 163.4 (C═N) ppm. MS (EI, 70 eV): m/z (%) = 412 (2%) $[M]^+$; Anal. for $C_{22}H_{20}N_8O$ (412.45): calcd. C, 64.07; H, 4.89; N, 27.17; found: C, 64.07; H, 4.89; N, 27.17.

*3-((5-Azido-3-methyl-1-phenyl-1H-pyrazol-4-yl) methyleneamino)-2-thioxothiazolidin-4-one (**19d**)*

Product **19d** was separated as red crystals, yield 78%; m. p. 202°C to 204°C. IR (KBr): $\tilde{\nu}$ = 2212 (N_3), 1673 (C═O), 1598, 1526 (C═N), 1071 (C═S), 694 (C-S) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.44 (s, 3 H, CH_3), 4.13 (s, 2 H, CH_2),7.29-8.08 (m, 9 H, CH_{arom}), 9.32 (s, 1 H, CH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.3 (CH_3), 40.3 (CH_2), 105.3, 142.6 (C═C), 123.9, 126.8, 129.4, 137.1, 138.8 (aromatic, C─ H), 143.9, 149.4 (C═N), 172.1 (C═O), 197.1 (C═S) ppm. MS (EI, 70 eV): m/z (%) = 357 (10%) $[M]^+$; Anal. for $C_{14}H_{11}N_7OS_2$ (357.41): calcd. C, 47.05; H, 3.10; N, 27.43; S, 17.94; found: C, 47.10; H, 3.08; N, 27.40; S, 17.98.

5 | BIOASSAY MATERIALS

The used cell lines and bacteria were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). RPMI 1640 medium, fetal bovine serum (FBS), trypan blue, penicillin G, and streptomycin were obtained from Gibco BRL (Gaithersburg, MD, USA). Mueller Hinton Broth was obtained for HIMEDIA Laboratories (India). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and doxorubicin were obtained from Sigma-Aldrich (St Louis, MO, USA).

5.1 | Cytotoxic assay (MTT proliferation assay)

The cytotoxic activity of the prepared derivatives was evaluated using MTT proliferation assay. Cells were seeded at 4×10^4 per well in 96-well culture plates before treatment with different concentrations of the tested compound. After treatment for 72 hours, the cytotoxicity of the tested compound was determined using the MTT cell proliferation assay (thiazolyl blue tetrazolium bromide, Sigma-M2128). Light absorbance values ($OD = OD_{570} - OD_{620}$) were recorded at 570 and 620 nm using an ELISA reader (Anthoslabtec Instrument, Salzburg, Austria) for calculating the concentration that caused 50% inhibition (IC_{50}), ie, the cell concentration at which the light absorbance value of the experimental group is half that of the control group. These results were expressed as a percentage of the control ± SD established from n = 4 wells per experiment from three independent experiments.[29,30]

5.2 | MIC tests

The minimum inhibitory concentration (MIC) test was performed by means of broth dilution method in a 96-well microtiter plate (https://www.nature.com/articles/ncomms13803#t1). Test compound was diluted with DMSO in the range from 512 to 1 μg/mL and using Streptomycin and Penicillin G as a standard antibiotic. A series of two-fold dilution of sample was dispersed in the columns of wells. Finally, 100 μL of bacterial suspension prepared equivalent to 0.5 McFarland units with sterile saline was added to each well and incubated at 37°C for 24 hours and recorded at 600 nm using an ELISA reader (Anthoslabtec Instrument, Salzburg, Austria).[31–33]

6 | CONCLUSION

A series of azidopyrazole derivatives was prepared by the reaction of 5-azido-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (azidopyrazole) with primary amines and methylene compounds. The prepared compounds were obtained in medium to good yields. The reaction mechanism proceeded through the formation of an imine derivative. The evaluation of the cytotoxic activity of some of the prepared compounds indicated that 3-((5-azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methylene)pentane-2,4-dione (**5b**) was the most active compound against Molt 4, U937, Sup-T1, and 562 cells with IC_{50} 10.45, 18.47, 8.41, and 10.56 μg/mL, respectively, after 72 hours. The prepared compounds showed weak or no activity *S. aureus*, *E. coli*, and *P. aeruginosa*, suggesting selective toxicity towards cancer cell lines.

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