Microwave-assisted one pot three-component synthesis of some novel pyrazole scaffolds as potent anticancer agents

Sobhi M. Gomha1*, Mastoura M. Edrees2,3, Rasha A. M. Faty1, Zeinab A. Muhammad2 and Yahia N. Mabkhot4

Abstract

Background: Pyrazoles, thiazoles and 1,3,4-thiadiazoles have been reported to possess various pharmacological activities.

Results: An efficient and a novel approach for the synthesis of some novel pyrazole based-azoles are described via multi-component reaction under controlled microwave heating conditions. The structures of the synthesized compounds were assigned on the basis of elemental analysis, IR, 1H NMR and mass spectral data. All the synthesized compounds were tested for in vitro activities against two antitumor cell lines, human lung cancer and human hepatocellular carcinoma compared with the employed standard antitumor drug (cisplatin).

Conclusions: All the newly synthesized compounds were evaluated for their anticancer activity against human lung cancer and human hepatocellular carcinoma cell lines using MTT assay. The results obtained exploring the high potency of six of the tested compounds compared with cisplatin.

Keywords: Acetylpyrazoles, Enaminones, Hydrazonoyl chlorides, Thiazoles, Thiadiazoles, Anticancer activity

Background

Multi-component reactions (MCR) are one-pot processes with at least three components to form a single product, which incorporates most or even all of the starting materials [1–6]. The huge interest for such multi-component reactions during the last years has been oriented towards developing combinatorial chemistry procedures, because of their high efficiency and convenience of these reactions in comparison with multistage procedures. Also, the utility of MCR under microwave irradiation in synthesis of heterocyclic compounds enhanced the reaction rates and improve the regioselectivity [7–12].

On the other hand, pyrazole and its derivatives have drawn considerable attention of the researchers in the past few decades owing to their high therapeutic values. Some of the drugs, possessing pyrazole as basic moiety, like celecoxib [13], deracoxib [14], etoricoxib and atorvastatin [15] are already booming in the market. Pyrazole derivatives possess an extensive range of pharmacological activities such as anti-inflammatory, antipyretic, analgesic, antimicrobial, sodium channel blocker, antitubercular, antiviral, antihypertensive, antiglaucoma, antioxidant, antidepressant, anxiolytic, neuroprotective and antidiabetic activity [16–23]. Furthermore, pyrazole prodrugs have also been reported to possess significant anticancer activities [24–30]. Keeping this in mind, and in continuation of our previous work on the synthesis of new anticancer agents [31–40], we herein present an efficient regioselective synthesis of novel 4-heteroaryl-pyrazoles, which have not been reported hitherto in a multicomponent synthesis under microwave irradiation and to assess their anticarcinogenic effects against hepatocellular carcinoma (HepG-2) and human lung cancer (A-549) cell lines.

*Correspondence: s.m.gomha@gmail.com
1 Department of Chemistry, Faculty of Science, Cairo University, Giza 12613, Egypt
Full list of author information is available at the end of the article

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Results and discussion

Chemistry

Multi-component reaction of acetyl pyrazole 1 [41], dimethylformamide dimethylacetal (DMF–DMA) 2 and nitrileimine 4a–d (generated in situ from 3a–d with triethylamine) in toluene under conventional heating for 10–15 h or under microwave irradiation at 150 °C for 4–10 min. afforded compound 6a–d rather than its isomeric structure 8a–d in 66–70 and 84–90%, respectively (Scheme 1; Table 1). The structure of 6a–d was confirmed by their spectral data (IR, MS and 1H-NMR) and elemental analyses. For example, the IR spectra of products 6 revealed in each case two absorption bands in the regions υ 1638–1676 and 1682–1724 cm⁻¹ due to the two carbonyl groups. The 1HNMR spectra showed, in addition to the expected signals for the aromatic protons, three singlet signals at δ ~2.34, 2.55 and 8.92 revealed to the two methyl groups and the pyrazole-H5, respectively. The mass spectra of products 6a–d revealed a molecular ion peak for each one which is consistent with the respective molecular weight. These data are much closer to those reported in literature on similar work [42–44].

Compound 6a was alternatively synthesized by reacting enamino 9 (prepared separately via condensation of acetyl pyrazole 1 with DMF–DMF) with 2-oxo-N-phenylpropanehydrazonoyl chloride (3a) in toluene containing catalytic amount of TEA under MWI. The obtained product was found to be identical with 6a in all respects (TLC, mp and IR spectrum) which affords further evidence to all structures 6a–d. The latter products were assumed to be formed via initial 1,3-dipolar cycloaddition of the nitrileimines 4a–d to the activated double bond in enamino 9 to afford the non-isolable cycloadducts 5 which underwent loss of dimethylamine yielding the final pyrazole derivatives 6a–d.

The results obtained Table 1 indicate that, unlike classical heating, microwave irradiation results in higher yields and shorter reaction times for all the carried reactions. Microwave irradiation facilitates the polarization of the molecules under irradiation causing rapid reaction to occur. This is consistent with the reaction mechanism, which involves a polar transition state [45].

By the same way reaction of acetyl pyrazole 1 with nitrile-oxide 11a, b (derived from reaction of hydroxymoiy chloride 10a, b with TEA) and DMF–DMA in toluene under microwave irradiation at 150 °C gave isoxazoles 13a, b (Scheme 2; Table 1). The 1H NMR spectrum of the product revealed a singlet signal at 9.67 ppm assigned for isoxazole-5H proton not isoxazole-4H proton [42–44, 46] which consistent with the isomeric structure 13 rather than the isomeric structure 15. Moreover, the mass spectrum of 13a and 13b revealed a molecular ion peaks at m/z = 506 and 446, respectively, which is consistent with their molecular weights.

Furthermore, alternative synthesis of compound 13a was achieved via reaction enamino 9 with N-hydroxy-2-naphthimidoyl chloride (10a) under the same reaction condition to yield authentic product 13a (Scheme 2).

Next, our study was extended to investigate the reactivity of compound 1 towards thiosemicarbazide and various hydrazonoyl halides aiming to synthesize new pyrazole based—1,3-thiazoles and 1,3,4-thiadiazoles. Thus, acetyl pyrrole 1, thiosemicarbazide 2 and α-keto hydrazonoyl halides 3a, b, e were allowed to react in a one-pot three-component reaction in dioxane containing catalytic amount of TEA under MWI to afford the aryloxothiazole derivatives 18a–c, respectively (Scheme 3; Table 1). The reaction goes in parallel to literature [32, 35–37].

The structure of the products 18a–c was assigned based on the spectral data and elemental analyses. For example mass spectrum of compound 18a revealed molecular ion peak at m/z 542 and its 1H NMR spectrum exhibited four characteristic singlet signals at 2.32, 2.36, 2.48 and 10.47 assignable to three CH₃ groups and NH protons, respectively, in addition to an aromatic multiplet in the region 6.99–7.93 ppm equivalent to 12 protons. Its IR spectra showed one NH group band at 3396 cm⁻¹.

The structure of products 18 was further confirmed by an alternative method. Thus, reaction of acetylpyrazole 1 with thiosemicarbazide 16 under MWI in ethanol containing drops of concentrated HCl led to the formation of product 19. Compound 19 was then react with 2-oxo-N-phenylpropanehydrazonoyl chloride (3a) in dioxane containing catalytic amount of TEA under MWI to give a product identical in all respects (IR, mp and mixed mp.) with 18a (Scheme 3).

In a similar manner, when acetyl pyrazole 1 was allowed to react with thiosemicarbazide 2 and ethyl (N-arylhydrazono)-chloroacetates 3c, f in dioxane in the presence of triethylamine under MWI, it afforded in each case a single isolable product, namely, 2-(2-(1-(5-methyl-1-(4-nitrophenyl)-3-(thiophen-2-yl)-1H-pyrazol-4-yl) ethylidene) hydrazinyl)-5-(2-arylhydrazono) thiazol-4(5H)-one 21a, b (Scheme 4; Table 1). Structure 21 was confirmed by elemental analysis, spectral data (IR, 1H NMR, and mass), and alternative synthesis route. Thus, thiosemicarbazone 19 was reacted with ethyl-1-2-chloro-2-(2-phenylhydrazono)acetate (3c) in dioxane in the presence of TEA under MWI afforded a product identical in all aspects (mp, mixed mp, and spectra) with 21a (Scheme 4).

Finally, the reactivity of acetylpyrazole 1 towards hydrazonoyl halides, be bereft of a-keto group, was
examined. In the present study, we have established that reaction of acetylpyrazole 1 with N-thiosemicarbazide 16 and aryl carbohydrazonoyl chlorides 3d, g gave the respective 1,3,4-thiadiazoles 23a, b as the end products (Scheme 5; Table 1). The structures of compounds 23a, b were confirmed on the bases of spectral data and elemental analyses (see Experimental part). The reaction proceeded via S-alkylation, with removal of hydrogen chloride, to give S-alkylated intermediates 22 followed by intramolecular Michael type addition under
the employed reaction conditions, followed by elimination of ammonia, afforded the final product 23 [36, 47] (Scheme 5).

Cytotoxic activity

The in vitro growth inhibitory activity of the synthesized compounds 6a–d, 9, 13a, b, 18a–c, 19, 21a, b and 23a, b was investigated against two carcinoma cell lines: human lung cancer (A-549) and human hepatocellular carcinoma (HepG-2) in comparison with the well-known anticancer standard drug (cisplatin) under the same conditions using colorimetric MTT assay. Data generated were used to plot a dose response curve of which the concentration of test compounds required to kill 50% of cell population (IC50) was determined. The results revealed that all the tested compounds showed inhibitory activity to the tumor cell lines in a concentration dependent manner. Interestingly, the results represented in Table 2 and Fig. 1 showed that compounds 13a, b and 21a were the most active compounds (IC50 value of 4.47 ± 0.3, 3.46 ± 0.6, 3.10 ± 0.8 μg/mL, respectively) against the lung carcinoma cell line (A549), compared with cisplatin reference drug with IC50 value of 0.95 ± 0.23 μg/mL. Moreover, the order of activity against A549 cell line was 18c > 18b > 19 > 9 > 6a > 6c > 23b > 6d > 18a > 21b > 6b.

Table 1 Comparative data of conventional (A) and MW (B) methods for the synthesis of compounds 6a–d, 13a, b, 18a–c, 21a, b and 23a, b

| Compound no. | Conventional method (A) | Microwave method (B) |
|--------------|-------------------------|----------------------|
|              | Time (h) | Yield (%) | Time (min) | Yield (%) |
| 6a           | 12       | 66        | 4          | 84        |
| 6b           | 15       | 68        | 10         | 85        |
| 6c           | 10       | 70        | 8          | 88        |
| 6d           | 8        | 69        | 5          | 90        |
| 13a          | 12       | 67        | 6          | 82        |
| 13b          | 10       | 70        | 6          | 89        |
| 18a          | 8        | 66        | 7          | 90        |
| 18b          | 6        | 68        | 10         | 88        |
| 18c          | 4        | 67        | 7          | 90        |
| 21a          | 6        | 69        | 8          | 86        |
| 21b          | 5        | 64        | 6          | 92        |
| 23a          | 8        | 72        | 10         | 81        |
| 23b          | 8        | 67        | 9          | 83        |

On the other hand, compounds 6a, 9, 13b, 23b were the most active compounds (IC50 value of 5.60 ± 0.8, 5.67 ± 1.2, 4.47 ± 0.9 and 5.67 ± 1.2 μg/mL, respectively) against liver carcinoma cell line (HepG2) cell line while the rest compounds have moderate activities.

Experimental

Chemistry

General

Melting points were measured on an Electrothermal IA 9000 series digital melting point apparatus (Bibby Sci. Lim. Stone, Staffordshire, UK). IR spectra were measured on PyeUnicam SP 3300 and Shimadzu FTIR 8101 PC infrared spectrophotometers (Shimadzu, Tokyo, Japan) in potassium bromide discs. NMR spectra were measured on a Varian Mercury VX-300 NMR spectrometer (Varian, Inc., Karlsruhe, Germany) operating at 300 MHz (1H-NMR) and run in deuterated dimethyl-sulfoxide (DMSO-d6). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer (Tokyo, Japan) at 70 eV. Elemental analyses were measured by using a German made Elementarvario LIII CHNS analyzer. Antitumor activity of the products was measured at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt. Hydrazonoyl halides 3a–g were prepared following literature method [41, 48].

Synthetic procedures

Synthesis of trisubstituted pyrazoles 6a–d and isoxazoles 13a,b

Method A To a stirred solution of acetyl pyrazole 1 (0.327 g, 1 mmol), dimethylformamide dimethylacetal 2 (1 mmol) and the appropriate hydrazonoyl halides 3a–d or hyroximoyl chlorides 10a, b (1 mmol) in dry toluene (15 mL), an equivalent amount of triethylamine (0.5 mL) was added. The reaction mixture was heated under reflux for 10–15 h (monitored through TLC). The precipitated triethylamine hydrochloride was filtered off, and the filtrate was evaporated under reduced pressure. The residue was triturated with MeOH. The solid product, so formed in each case, was collected by filtration, washed with water, dried, and crystallized from the proper solvent to afford the corresponding pyrazole 6a–d and isoxazole derivatives 13a, b, respectively.

Method B Repetition of the same reactions of method A with heating in microwave oven at 500 W and 150 °C for 4–10 min., gave products identical in all respects with those separated from method A. The products 6a–d and 13a, b together with their physical constants are listed below.

1-(4-(5-Methyl-1-(4-nitrophenyl)-3-(thiophen-2-yl)-1H-pyrazole-4-carbonyl)-1-phenyl-1H-pyrazol-3-yl)ethanone (6a) Brown solid, mp 208–210 °C; IR (KBr) νmax 1599 (C=N), 1670, 1682 (2C=O), 2924, 3105 (C–H) cm⁻¹; 1H NMR (DMSO-d6) δ 2.34 (s, 3H, CH₃), 2.55 (s, 3H, CH₃), 6.98–8.39 (m, 12H, Ar–H), 8.92 (s, 1H, pyrazole-H5); MS m/z (%) 497 (M⁺, 9), 342 (25), 252 (22), 174 (11), 145 (22),
115 (26), 103 (40), 76 (100), 63 (13), 50 (19). Anal. Calcd. for C\textsubscript{26}H\textsubscript{19}N\textsubscript{5}O\textsubscript{4}S (497.53): C, 62.77; H, 3.85; N, 14.08. Found: C, 63.08; H, 3.55; N, 13.70%.

1-(4-(5-Methyl-1-(4-nitrophenyl)-3-(thiophen-2-yl)-1H-pyrazole-4-carbonyl)-1-(p-tolyl)-1H-pyrazol-3-yl)ethane (6b) Yellow solid, mp 222–224 °C; IR (KBr) \( \nu \) max 1597 (C=N), 1676, 1688 (2C=O), 2919, 3118 (C–H) cm\(^{-1}\); \(^{1}\)H NMR (DMSO-\( d_6 \)) \( \delta \) 2.24 (s, 3H, CH\(_3\)), 2.34 (s, 3H, CH\(_3\)), 2.56 (s, 3H, CH\(_3\)), 7.12 (t, \( J = 1.2 \) Hz, 1H, thiophene-H), 7.31 (d, \( J = 1.2 \) Hz, 1H, thiophene-H), 7.33 (d, \( J = 1.2 \) Hz, 1H, thiophene-H), 7.55 (d, \( J = 4.4 \) Hz, 2H, Ar–H), 7.63 (d, \( J = 4.4 \) Hz, 2H, Ar–H), 7.88 (d, \( J = 8.8 \) Hz, 2H, Ar–H), 8.39 (d, \( J = 8.8 \) Hz, 2H, Ar–H), 10.58 (s, 1H, pyrazole-H5); \(^{13}\)C-NMR (DMSO-\( d_6 \)) \( \delta \) 13.3, 20.8, 25.7 (CH\(_3\)), 115.3, 117.6, 118.9, 121.37, 122.7, 125.2, 126.7, 128.1, 129.4, 130.1, 132.2, 133.8, 138.1, 140.6, 143.43, 144.4, 146.8, 147.2 (Ar–C and C=N), 188.2, 194.9 (C=O); MS m/z (%) 511 (M\(^+\)), 2, 406 (10), 266 (6), 219 (11), 168 (7), 147 (7), 125 (11), 104 (25), 98 (17), 83 (93), 79 (44), 69 (35), 54 (53), 44 (100). Anal. Calcd. for C\textsubscript{27}H\textsubscript{21}N\textsubscript{5}O\textsubscript{4}S (511.55): C, 63.58; H, 4.14; N, 13.69. Found: C, 63.78; H, 4.05; N, 13.29%.

Ethyl 4-(5-methyl-1-(4-nitrophenyl)-3-(thiophen-2-yl)-1H-pyrazole-4-carbonyl)-1-phenyl-1H-pyrazole-3-carboxylate (6c) Yellow solid, mp 207–209 °C; IR (KBr) \( \nu \) max 15984 (C=N), 1660, 1724 (2C=O), 2931, 2974 (C–H) cm\(^{-1}\); \(^{1}\)H NMR (DMSO-\( d_6 \)) \( \delta \) 2.24 (t, \( J = 7.6 \) Hz, 2H, CH\(_2\)CH\(_3\)), 2.34 (s, 3H, CH\(_3\)), 2.47 (q, \( J = 7.1 \) Hz, 2H, CH\(_2\)CH\(_3\)), 6.96–8.43 (m, 12H, Ar–H), 8.99 (s, 1H, pyrazole-H5); MS m/z (%) 527 (M\(^+\)), 6, 484 (22), 366 (26), 328 (33), 268 (50), 226 (35), 210 (37), 151 (49), 124 (78), 115 (61), 75 (100), 42 (45). Anal. Calcd. for C\textsubscript{27}H\textsubscript{21}N\textsubscript{5}O\textsubscript{5}S (527.55): C, 61.47; H, 4.01; N, 13.28. Found: C, 61.77; H, 3.75; N, 12.94%.
(5-Methyl-1-(4-nitrophenyl)-3-(thiophen-2-yl)-1H-pyrazol-4-yl)(1-(4-nitrophenyl)-3-(thiophen-2-yl)-1H-pyrazol-4-yl)methanone (6d)  Orange solid, mp 219–220 °C; IR (KBr) νmax 1595 (C=N), 1638 (C=O), 2924, 3105 (C–H) cm⁻¹; ¹H NMR (DMSO-d₆) δ 2.34 (s, 3H, CH₃), 6.98–8.52 (m, 14H, Ar–H), 9.28 (s, 1H, pyrazole-H5); ¹³C-NMR (DMSO-d₆): δ 26.9 (CH₃), 113.1, 113.3, 115.0, 115.6, 122.5, 122.6, 123.1, 123.6, 126.5, 126.7, 128.4, 131.1, 131.7, 132.1,
**Scheme 5** Synthesis of thiadiazoles 23a,b

Table 2 The in vitro inhibitory activity of tested compounds against tumor cell lines expressed as IC50 values (μg/mL) ± standard deviation from three replicates

| Tested compounds | R     | Ar′    | A-549 IC50 ± SD | HepG2 IC50 ± SD |
|------------------|-------|--------|-----------------|-----------------|
| 6a               | COCH₃ | Ph     | 22.9 ± 0.9      | 5.60 ± 0.8      |
| 6b               | COCH₃ | 4-MeC₆H₄| 38.5 ± 1.2      | 44.4 ± 1.3      |
| 6c               | COOEt | Ph     | 23.3 ± 0.9      | 22.4 ± 0.9      |
| 6d               | 2-Thienyl | 4-NO₂C₆H₄| 30.6 ± 1.1      | 35.9 ± 1.4      |
| 9                | –     | –      | 22.6 ± 0.8      | 5.67 ± 1.2      |
| 13a              | –     | 2-Naphthyl | 4.47 ± 0.3      | 8.03 ± 1.1      |
| 13b              | –     | 2-Furyl | 3.46 ± 0.6      | 4.67 ± 0.9      |
| 18a              | –     | Ph     | 32.7 ± 1.2      | 22.4 ± 1.1      |
| 18b              | –     | 4-MeC₆H₄| 19.1 ± 1.1      | 6.67 ± 1.3      |
| 18c              | –     | 4-ClC₆H₄| 18.2 ± 0.9      | 21.8 ± 0.9      |
| 19               | –     | –      | 21.3 ± 0.8      | 23.1 ± 1.1      |
| 21a              | –     | Ph     | 3.10 ± 0.8      | 23.9 ± 1.1      |
| 21b              | –     | 4-MeC₆H₄| 33.6 ± 0.9      | 43.4 ± 0.8      |
| 23a              | 2-Thienyl | 4-NO₂C₆H₄| 27.9 ± 1.1      | 34.4 ± 0.9      |
| 23b              | Ph    | Ph     | 23.4 ± 1.2      | 5.67 ± 1.7      |
| Cisplatin        | –     | –      | 0.95 ± 0.23     | 1.4 ± 0.37      |
132.3, 136.5, 137.1, 141.5, 141.6, 142.4, 142.6, 142.8 (Ar–C and C=N), 197.2 (C=O); MS m/z (%) 582 (M+, 6), 532 (12), 383 (16), 286 (11), 219 (21), 135 (49), 79 (16), 83 (27), 76 (67), 60 (28), 45 (100). Anal. Calcd. for C28H18N6O5S2 (582.61): C, 57.72; H, 3.11; N, 14.42. Found: C, 57.99; H, 2.80; N, 14.12%.

Synthesis of 3-(dimethylamino)-1-(5-methyl-1-(4-nitroph enyl)-3-(thiophen-2-yl)-1H-pyrazol-4-yl)prop-2-en-1-one (9).

A mixture of acetyl pyrazole 1 (3.27 g, 10 mmol) and dimethylformamide–dimethylacetal (DMF–DMA) (10 mmol) in dry toluene (20 mL) was refluxed in micro-wave oven at 500 W and 150 °C for 5 min., then left to cool to room temperature. The precipitated product was filtered off, washed with light petroleum (40–60 °C), and dried. Recrystallization from benzene afforded enaminone 1 as orange solid, mp 250–252 °C; IR (KBr) ν max 1597 (C=N), 1660 (C=O), 2976, 3117 (C–H) cm−1; 1H NMR (DMSO-d6) δ 2.31 (s, 3H, CH3), 7.13–8.45 (m, 14H, Ar–H), 9.67 (s, 1H, isoxazole-H5); 13C-NMR (DMSO-d6): δ 26.9 (CH3), 110.0, 113.3, 115.0, 115.1, 115.5, 122.5, 123.3, 124.5, 125.0, 126.5, 126.7, 128.4, 130.8, 133.6, 135.4, 136.9, 137.0, 141.5, 141.6, 142.6, 148.8, 152.4, 160.0 (Ar–C and C=N), 188.3 (C=O); MS m/z (%) 506 (M+, 2), 435 (9), 412 (14), 379 (45), 214 (12), 142 (10), 105 (26), 93 (21), 77 (51), 65 (62), 60 (52), 43 (100). Anal. Calcd. for C28H18N4O4S (506.53): C, 66.39; H, 3.58; N, 11.06. Found: C, 66.04; H, 3.21; N, 10.86%.

(5-Methyl-1-(4-nitrophenyl)-3-(thiophen-2-yl)-1H-pyrazol-4-yl)(3-(naphthalen-2-yl)isoxazol-4-yl)methanone (13a) Yellow solid, mp 203–205 °C; IR (KBr) ν max 1597 (C=N), 1660 (C=O), 2976, 3117 (C–H) cm−1; 1H NMR (DMSO-d6) δ 2.31 (s, 3H, CH3), 7.13–8.45 (m, 14H, Ar–H), 9.67 (s, 1H, isoxazole-H5); 13C-NMR (DMSO-d6): δ 26.9 (CH3), 110.0, 113.3, 115.0, 115.1, 115.5, 122.5, 123.3, 124.5, 125.0, 126.5, 126.7, 128.4, 130.8, 133.6, 135.4, 136.9, 137.0, 141.5, 141.6, 142.6, 148.8, 152.4, 160.0 (Ar–C and C=N), 188.3 (C=O); MS m/z (%) 506 (M+, 2), 435 (9), 412 (14), 379 (45), 214 (12), 142 (10), 105 (26), 93 (21), 77 (51), 65 (62), 60 (52), 43 (100). Anal. Calcd. for C28H18N4O4S (506.53): C, 66.39; H, 3.58; N, 11.06. Found: C, 66.04; H, 3.21; N, 10.86%.

(3-(Furan-3-yl)isoxazol-4-yl)(5-methyl-1-(4-nitrophenyl)-3-(thiophen-2-yl)-1H-pyrazol-4-yl)methanone (13b) Orange solid, mp 209–211 °C; IR (KBr) ν max 1598
(C=3N), 1664 (C=O), 2925, 3107 (C–H) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.34 (s, 3H, CH$_3$), 7.13–8.61 (m, 10H, Ar–H), 9.23 (s, 1H, pyrazole-H5); MS m/z (%) 446 (M$^+$, 2), 392 (100), 349 (43), 317 (23), 285 (11), 234 (16), 191 (16), 172 (20), 130 (26), 77 (69). Anal. Calcd. for C$_{22}$H$_{14}$N$_8$O$_2$S$_2$ (446.44): C, 59.19; H, 3.16; N, 12.55. Found: C, 59.50; H, 2.80; N, 12.17%.

**Alternate synthesis of 6a and 13a** Equimolar amounts of enamnine 9 (0.382 g, 1 mmol) and hydrazonoyl halide 3a or hyroximoyl chloride 10a (1 mmol) in dry toluene (15 mL) containing an equivalent amount of triethylamine (0.5 mL) was refluxed in microwave oven at 500 W and 150 °C for 6 min., gave products identical in all respects (mp, mixed mp and IR spectra) with compounds 6a and 13a, respectively.

**Synthesis of thiazoles 18a-c and 21a, b and thiadiazoles 23a, b** *Method A* To a stirred solution of acetyl pyrazole 1 (0.327 g, 1 mmol), thiosemicarbazide 16 (0.091 g, 1 mmol) and the appropriate hydrazonoyl halides 3a, b, e or 3c, f for 3d, g (1 mmol) in dioxane (15 mL), an equivalent amount of triethylamine (0.5 mL) was added. The reaction mixture was heated under reflux for 4–8 h (monitored through TLC). Excess of solvent was removed under reduced pressure and the reaction mixture was triturated with MeOH. The product separated was filtered, washed with MeOH, dried and recrystallized from the proper solvent to give thiazoles 18a–c and 21a, b and thiadiazoles 23a, b, respectively.

**Method B** Repetition of the same reactions of method A with heating in microwave oven at 500 W and 150 °C for 4–10 min., gave products identical in all respects with those separated from method A. The products 18a–c, 21a, b and 23a, b together with their physical constants are listed below.

4-Methyl-2-{2-(1-(5-methyl-1-(4-nitrophenyl)-3-(thiophen-2-yl)-1H-pyrazol-4-yl)ethylidene) hydrazinyl]-5-(phenyldiazenyl)thiazole (18a) Orange solid, mp 219–220 °C; IR (KBr) $\nu_{max}$ 1509 (C=CN), 2974 (C–H), 3396 (NH) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.32 (s, 3H, CH$_3$), 2.36 (s, 3H, CH$_3$), 2.48 (s, 3H, CH$_3$), 6.99–7.93 (m, 12H, Ar–H), 10.65 (s, 1H, NH); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ 9.2, 12.5, 24.6 (CH$_3$), 114.5, 121.4, 123.1, 125.2, 126.3, 127.0, 127.9, 128.1, 128.5, 128.9, 135.3, 140.4, 140.9, 143.1, 144.1, 145.3, 145.79, 153.3, 163.4 (Ar–C and C–N); MS m/z (%) 542 (M$^+$, 6), 432 (16), 253 (13), 138 (11), 106 (69), 90 (12), 78 (100), 64 (11), 51 (34). Anal. Calcd. for C$_{26}$H$_{22}$N$_8$O$_2$S$_2$ (542.64): C, 57.55; H, 4.09; N, 19.80%.

5-((4-Chlorophenyl)diazenyl)-4-methyl-2-(2-(1-(5-methyl-1-(4-nitrophenyl)-3-(thiophen-2-yl)-1H-pyrazol-4-yl)ethylidene)hydrazinyl)thiazole (18b) Orange solid, mp 226–228 °C; IR (KBr) $\nu_{max}$ 1600 (C=CN), 2924 (C–H), 3438 (NH) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.17 (s, 3H, CH$_3$), 2.32 (s, 3H, CH$_3$), 2.36 (s, 3H, CH$_3$), 2.47 (s, 3H, CH$_3$), 6.99–7.89 (m, 11H, Ar–H), 10.65 (s, 1H, NH); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ 12.0, 14.3, 15.7, 26.8 (CH$_3$), 105.3, 111.5, 114.9, 116.3, 117.9, 119.8, 120.8, 122.2, 126.4, 126.6, 127.9, 128.1, 131.9, 132.6, 137.6, 141.7, 142.1, 142.3, 170.2 (Ar–C and C–N); MS m/z (%) 556 (M$^+$, 18), 431 (18), 314 (25), 251 (43), 193 (32), 166 (29), 152 (43), 136 (20), 119 (45), 104 (67), 90 (68), 75 (100), 62 (55), 52 (28), 41 (41). Anal. Calcd. for C$_{27}$H$_{23}$N$_8$O$_2$S$_2$ (556.66): C, 58.26; H, 4.35; N, 20.13. Found: C, 58.58; H, 4.05; N, 19.80%.

**Synthesis of 2-(1-(5-methyl-1-(4-nitrophenyl)-3-(thiophen-2-yl)-1H-pyrazol-4-yl)ethylidene)hydrazinocarbothioamide (19)** Amixture of acetyl pyrazole 1 (3.27 g, 10 mmol) and thiosemicarbazide 16 (0.91 g, 10 mmol) in ethanol (20 mL) containing catalytic amounts of concentrated HCl was refluxed in microwave oven at 500 W and 150 °C for 6 min., then left to cool to room temperature. The precipitated product was filtered off, washed with ethanol, and dried. Recrystallization from acetic acid afforded thiosemicarbazone 19 as yellow solid, (78% yield), mp 212–215 °C; IR (KBr) $\nu_{max}$ 1596 (C=CN), 2926 (C–H), 3157, 3241, 3388 (NH and NH$_2$) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.17 (s, 3H, CH$_3$), 2.34 (s, 3H, CH$_3$), 7.10 (t, $J$ = 1.2 Hz, 1H, thiophene-H), 7.23 (d, $J$ = 1.2 Hz, 1H, thiophene-H), 7.56 (d, $J$ = 1.2 Hz, 1H, thiophene-H), 7.86 (d, $J$ = 8.8 Hz, 2H, Ar–H), 8.20 (s, 2H, NH$_2$), 8.38 (d, $J$ = 8.8 Hz, 2H, Ar–H), 10.28 (s, 1H, NH); MS m/z (%) 400 (M$^+$, 8), 322 (21), 284 (30), 211 (18), 176 (24), 150 (26), 130 (25), 112 (29), 105 (71), 97 (40), 83 (45), 69 (63), 57 (62), 43 (100). Anal. Calcd. for C$_{17}$H$_{16}$N$_8$O$_2$S$_2$ (400.48): C, 50.98; H, 4.03; N, 20.98. Found: C, 51.30; H, 3.73; N, 20.65%.
Alternate synthesis of thiazole 18a and 21a. Equimolar amounts of thiosemicarbazone 19 (0.400 g, 1 mmol) and hydrazonoyl chloride 3a or 3c (1 mmol) in dioxane (15 mL) containing an equivalent amount of triethylamine (0.05 mL) was refluxed in microwave oven at 500 W and 150 °C for 3 min., gave product identical in all respects (mp, mixed mp and IR spectra) with compounds 18a and 21a, respectively.

Biological activity
Anticancer activity
The cytotoxic evaluation of the synthesized compounds was carried out at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt according to the reported method [49].

Conclusion
In our present work, we herein present an efficient regioselective synthesis of novel 4-heteroaryl-pyrazoles, which have not been reported hitherto.

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