Synthesis, reactions and antitumour screening of new enaminones

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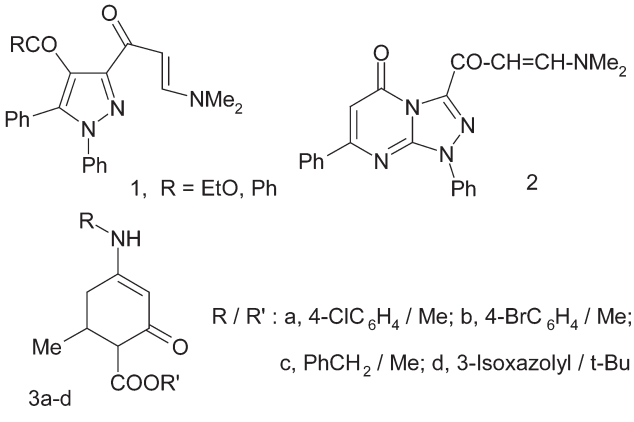
A new series of enaminones derived from 3-acetyl-1-aryl-4-benzoyl-5-phenyl-1*H*-pyrazoles has been obtained and their reactions with hydrazine hydrate, guanidine hydrochloride, 3-amino-1,2,4-triazole, 2-aminobenzimidazole and active methylenenitriles are described. The mechanisms and regioselectivity of the studied reactions are discussed. The results of screening of the antitumor activity of the enaminones against the human breast cancer cell line MCF-7 revealed that the compounds showed less activity than that of the reference drug doxorubicin. Their activity was found to depend on the nature of the substituent group on the 1-aryl moiety. The order of activity of the series is: H > 4–Cl > 4-MeO > 4-Me > 4-NO_2.

Keywords: enaminones, hydrazonoyl halides, dehydrative cyclisation, heterocycles

Recently, we reported that heterocyclic enaminones of types **1** and **2** (Scheme 1) each exhibited an unexpected activity against the human breast cancer cell line MCF-7.[1–3] Also, several reports indicate that cyclic enaminones of type **3a–d** (Scheme 1) proved to exhibit anticonvulsant properties and represent a new exciting series of compounds with efficacy in reducing seizures and hence are of great potential for the treatment of epilepsy.[4–11] In the light of these findings and in continuation of our recent work on the utility of enaminones for synthesis of hetaryl-pyrazoles,[1,2] it was thought interesting to synthesise new substituted enaminones **6** (Scheme 2) and explore their antitumor activity to shed some light upon structure activity relationships (SAR) and to study their reactions with some nitrogen and carbon nucleophiles.

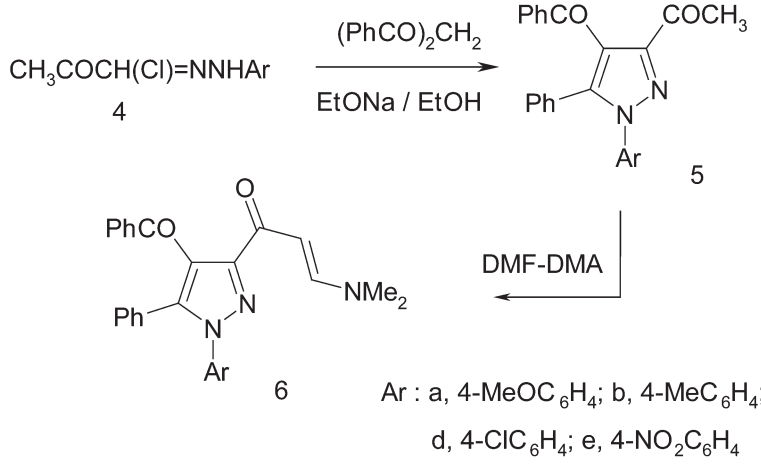


Scheme 1

Results and discussion

The starting precursors 4-benzoyl-1,5-diaryl-3-acetylpyrazoles **5a–e** were prepared by a literature method *via* reaction of the hydrazonoyl chlorides **4a–e** with dibenzoylmethane in ethanol in the presence of sodium ethoxide (Scheme 1).[3] Condensation of each of the pyrazoles **5a–e** with *N*,*N*-dimethylformamide-dimethylacetal (DMF-DMA) yielded the respective enaminones **6a–e** in 75–85% yield (Scheme 1). The structures of the enaminones **6a–e** were confirmed by their spectra (MS, IR and ^{1}H NMR (see Experimental). For example, their IR spectra revealed two carbonyl bands in the regions ν 1721–1668 and 1649–1642 cm^{-1}. Their ^{1}H NMR spectra showed, in each case, two doublet signals in the regions δ 5.84–5.86 and 7.86–7.22 with J = 13.0 Hz assignable to the two olefinic protons. This finding indicates also that such enaminones exist in the *trans*–Configuration depicted in Scheme 2. In addition the ^{1}H NMR spectra revealed two methyl protons singlet signals near δ 2.86 and 3.09.

Reactions of the enaminone **6c**, as a representative example of the series prepared, with some nitrogen and carbon nucleophiles were examined. Thus, treatment of **6c** with hydrazine hydrate in refluxing ethanol yielded a single product that was identified as 4-benzoyl-1,5-diphenyl-3-(pyrazol-3-yl)pyrazole **7** (Scheme 3). The structure of the latter was elucidated on the basis of its spectral data (IR, MS, and ^{1}H NMR) and elemental



Scheme 2

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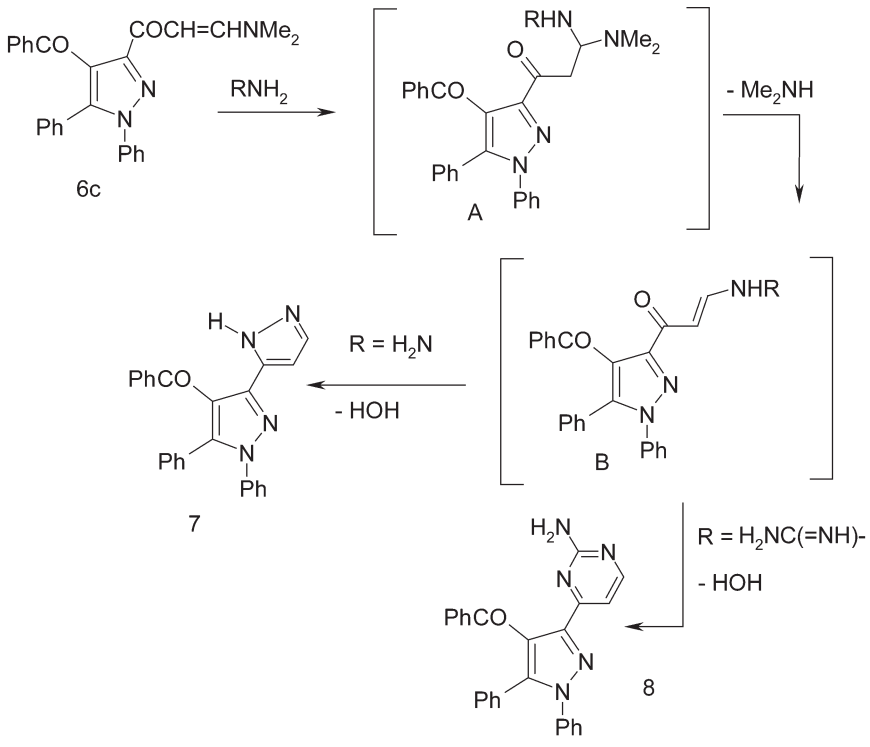


Scheme 3

analysis. For example, its ^{1}H NMR spectrum revealed a broad singlet (D_2O-exchangeable) at δ 12.9 due to the pyrazole-NH proton. This NH group displayed an absorption band at 3148 cm^{-1} in the IR spectrum of **7**. To account for the formation of **7**, it is suggested that the reaction starts with Michael-type addition of hydrazine to **6c** to give **A** which, in turn, eliminates dimethylamine to yield the intermediate **B**. The latter undergoes *in situ* dehydrative cyclisation to afford **7** as the end product (Scheme 3). This suggested pathway is consistent with literature reports on hydrazinolysis of other enaminones.[12]

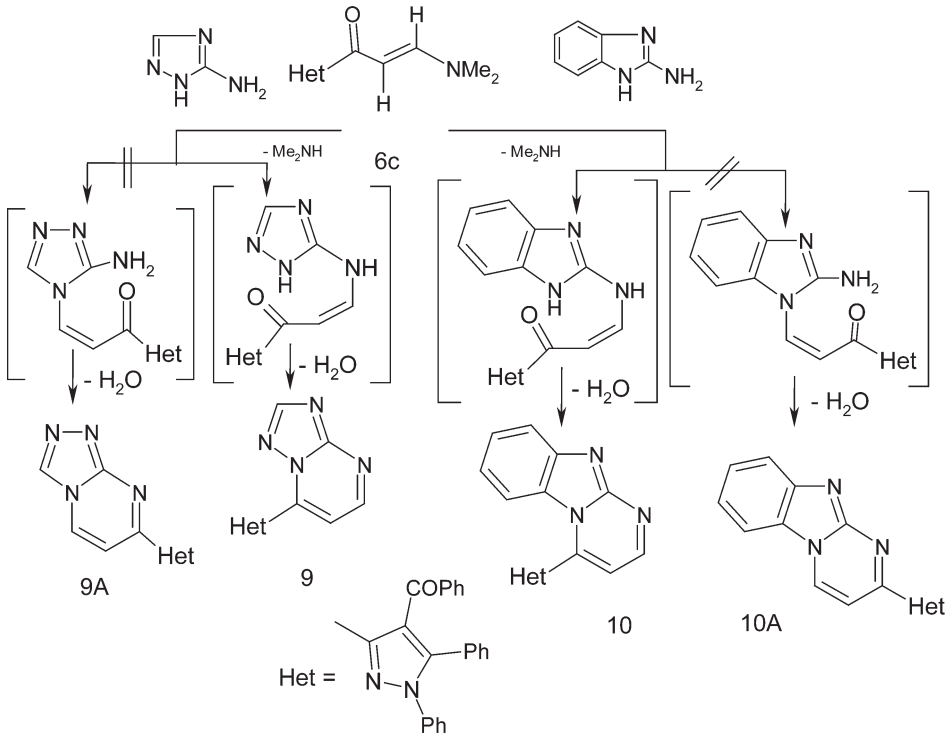
Also, treatment of the enaminone **6c** with guanidine hydrochloride in ethanol in the presence of sodium ethoxide afforded the pyrazolylpyrimidine derivative **8** (Scheme 2). This is consistent with literature reports which indicate that reactions of acyclic enaminones with guanidine afforded the respective 2-amino-4-substituted-pyrimidines.[14] The structure of the product **8** was assigned on the basis of its spectra (MS, IR and ^{1}H NMR) and elemental analysis (see Experimental).

Next, reactions of **6c** with 3-amino-1,2,4-triazole and 2-aminobenzimidazole were examined to shed some light on the regiochemistry of the reactions of **6** with heterocyclic amines. This is because reaction of **6c** with the former amine can possibly lead to the product **9** and/or **9A** whereas its reaction with the latter amine can give the product **10** and/or **10A** (Scheme 4). In our hands, reaction of **6c** with 3-amino-1,2,4-triazole, in ethanol in the presence of a catalytic amount of piperidine under reflux, was found to be regiospecific as it afforded only one product that was identified as 5-(4-benzoyl-1,5-diphenyl-1*H*-pyrazol-3-yl)-1,2,4-triazolo[1,5-*a*]pyrimidine **9** on the basis of its spectral data and elemental analysis. For example, its ^{1}H NMR spectrum revealed a one proton singlet at δ 8.46 and two doublet signals, each for one proton, at δ 7.58 and 8.84 with J = 4.5 Hz assignable to the H-2, H-6 and H-7 protons of the 1,2,4-triazolo[1,5-*a*]pyrimidine moiety, respectively.[13–17] The chemical shift value (δ 8.46) fully confirms that the condensation of **6c** with 3-amino-1,2,4-triazole provides the [1,5-*a*] isomer **9** rather than the [4,3-*a*] isomer **9A** in which the H-3 proton would resonate at δ *ca* 9.5 ppm.[16,17]

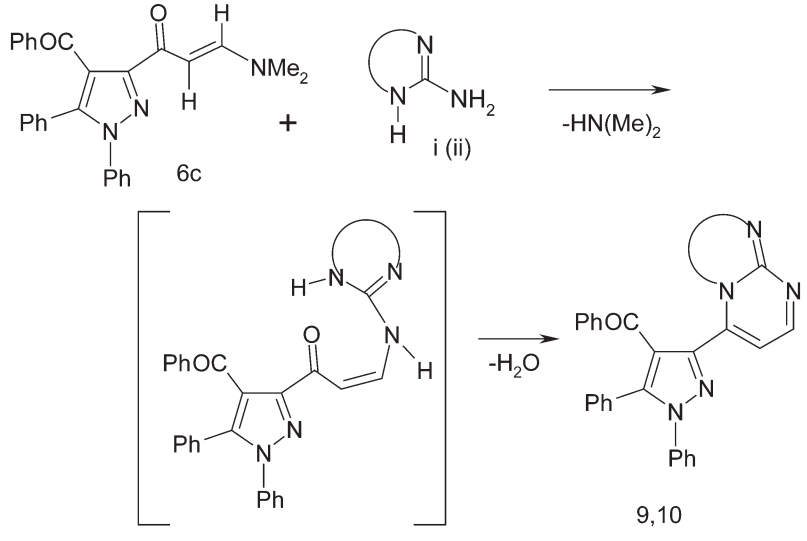
Reaction of **6c** with 2-aminobenzimidazole under the same reaction conditions was also found to be regiospecific as it afforded only one product, as evidenced by TLC analysis. On the basis of its spectra and elemental analysis, the isolated product was assigned the 4-(pyrazol-3-yl)pyrimido[1,2-*a*] benzimidazole structure **10** (Scheme 4). The other possible regioisomer namely 2-(pyrazol-3-yl)pyrimido[1,2-*a*]benzimidazole **10A** (Scheme 4) was discarded. The assignment of structure **10** was based on its spectral data (see Expeimental). For example, its ^{1}H NMR spectrum revealed two doublet signals, each for one proton, at δ 8.26 and 7.59 with J = 4.5 Hz which can be attributed to the H-2 and H-3 of the pyrimido [1,2-*a*]benzimidazole ring residue.[13,14] This finding is consistent with literature ^{1}H NMR data reported for 2- and 4-substituted pyrimido[1,2-*a*]benzimidazole isomers.[15] For example, while each isomer exhibits two doublet signals, the J value for the two doublets of the 2-substituted isomer is 7.0–7.5 Hz and for that of the 4-substituted one is 4.1–4.5 Hz.[15]

To account for the regioselective formation of the products **9** and **10**, it is suggested that the reactions started with Michael-type addition of the exocyclic amino group of each of the amines used, to the activated double bond of **6c** followed by *in situ* tandem elimination of dimethylamine and dehydrative cyclisation (Scheme 5).

Reactions of the enaminone **6c** with malononitrile **11a** and also with ethyl cyanoacetate **11b** in refluxing ethanol in the presence of sodium ethoxide, yielded products that were identified as the respective 6-(3-pyrazol-3-yl)pyridin-2(1*H*)-one derivatives **12a,b** (Scheme 5). The structures of the latter products were elucidated on the basis of their spectra (MS, IR and ^{1}H NMR) as well as their elemental analyses (see Experimental). Here also, it is suggested that the formation of **12** seems to start with Michael addition of the active methylene compound **11** to the activated double bond of **6c** followed by tandem cyclisation and elimination of dimethylamine to give the iminopyran intermediate. The latter intermediate undergoes *in situ*



Scheme 4



Scheme 5

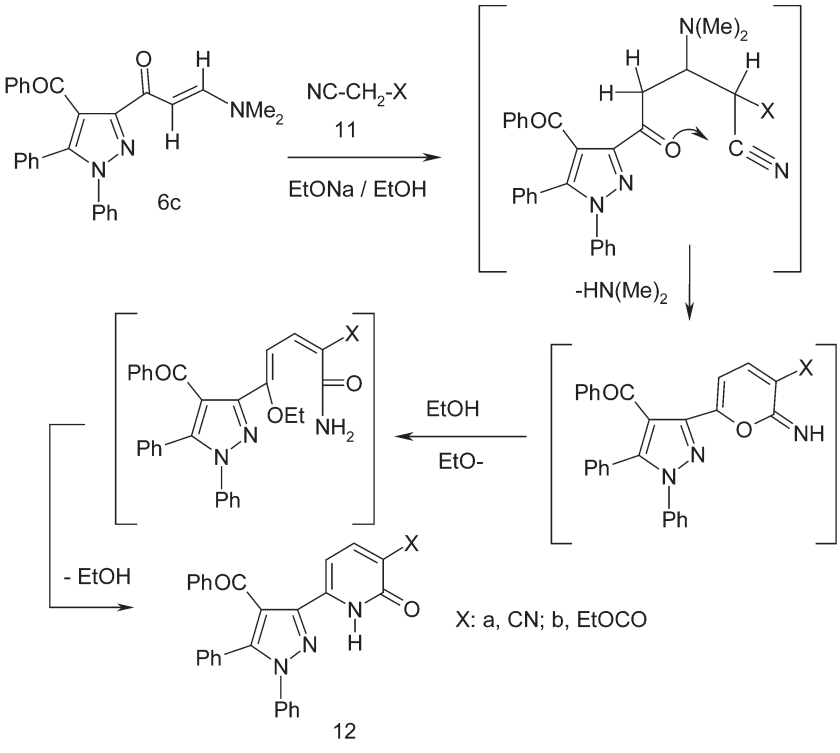
Dimroth type rearrangement *via* probably a base–Catalysed 1,6-addition of ethanol to the 2-iminopyran residue with subsequent electrocyclic ring opening, cyclisation and elimination of ethanol to give **12** as the final product (Scheme 6).[19]

The cytotoxic effects of the new enaminones **6a–e** against human breast cell line MCF-7 were evaluated at the National Institute of Cancer, Cairo, Egypt. Doxorubicin was used as a reference to evaluate the potency of the tested compounds. Five different concentrations of each compound and the reference were used in such screening tests and determination of IC_{50} values. The results are given in Table 1. As shown in this table, the compounds **6a,b,d,e** showed less activity than that of compound **6c**. This finding showed that both electron-donating and electron-withdrawing substituents increase the IC_{50} value and in turn decrease the antitumor activity. Furthermore the data indicate that the activity of the studied enaminones depends on the nature of the substituent group in the 1-aryl moiety. The order of activity of the studied series is thus: H > 4-Cl > 4-MeO > 4-Me > 4-NO_2.

Table 1 *In vitro* cytotoxic activity of the enaminones **6a–e**

Compd no.	Concentration, μg/mL					IC_{50} μg/mL
	0.0	5.0	12.5	25.0	50.0	
	Surviving fraction					
6a	1.00	0.673	0.284	0.210	0.141	8.33
6b	1.00	0.687	0.491	0.266	0.277	11.6
6c	1.00	0.261	0.168	0.076	0.070	0.87
6d	1.00	0.485	0.415	0.211	0.178	4.88
6e	1.00	0.739	0.541	0.225	0.264	13.4
Dox.[a]	1.00	0.261	0.267	0.277	0.272	0.70

[a] Doxotrobicin, an antitumour reference.



Scheme 6

Experimental

All melting points are uncorrected. IR spectra were recorded in KBr using Pye Unicam SP-1000 Spectrophotometer. ^{1}H NMR spectra were recorded for DMSO-d_6 solutions with TMS as internal reference using a Varian EM-200 MH*z* spectrometer. Mass spectra were recorded on AEI MS 30 mass spectrometer operating at 70eV. Elemental analyses were carried out at the Microanalytical Centre of Cairo University.

*Synthesis of 3-acetyl-1-aryl-4-benzoyl-5-phenyl-1H-pyrazoles (***5a–e***)*
To an ethanolic sodium ethoxide solution, prepared by dissolving sodium metal (0.12 g, 5 mmol) in absolute ethanol (20 mL), was added dibenzoylmethane (1.12 g, 5 mmol). To the resulting solution was added the appropriate *N*-phenyl 2-oxopropanehydrazonoyl chloride **4a** (5 mmol) portionwise while stirring the reaction mixture at room temperature. After complete addition, the reaction mixture was stirred for a further 3 h during which the hydrazonoyl chloride dissolved and a new solid precipitated. The latter was filtered off, washed and crystallised from ethanol to give the respective 3-acetyl-4-benzoyl-1-aryl-5-phenyl-1*H*-pyrazole **5**. The pyrazole derivatives **5a–e** prepared together with their physical constants are listed below.

*3-Acetyl-4-benzoyl-1-(4-methoxyphenyl)-5-phenyl-1*H*-pyrazole* (**5a**): Yellow crystals (yield 80%), m.p. 142–143°C. IR (KBr) ν 1680, 1670 (2 C=O); ^{1}H NMR (DMSO-d_6): δ 2.15 (s, 3H, $COCH_3$), 3.81 (s, 3H. OCH_3), 7.05–7.82 (m, 14H, ArH); MS: *m/z* (%): 397 (M^++1, 45), 396 (M^+, 2), 319 (40), 234 (100), 2107 (80), 105 (64), 77 (85). Anal. Calcd for $C_{25}H_{20}N_2O_3$ (396.45): C, 75.74; H, 5.08; N, 7.07. Found: C, 75.62; H, 5.10; N, 7.12%.

*3-Acetyl-4-benzoyl-1-(4-methylphenyl)-5-phenyl-1*H*-pyrazole* (**5b**): Yellow crystals (yield 80%), m.p. 152–153°C. IR (KBr) ν 1724, 1696 (2 C=O); ^{1}H NMR (DMSO-d_6): δ 2.13 (s, 3H, CH_3), 2.37 (s, 3H, $COCH_3$), 7.20–7.78 (m, 14H, ArH); MS: *m/z* (%): 381 (M^++1, 5), 380 (M^+, 8), 303 (92), 261 (60), 91 (100), 77 (94). Anal. Calcd for $C_{25}H_{20}N_2O_2$ (380.45): C, 78.93; H, 5.30; N, 7.36. Found: C, 78.74; H, 5.31; N, 7.51%.

*3-Acetyl-4-benzoyl-1,5-diphenyl-1*H*-pyrazole* (**5c**): Yellow crystals (yield 80%), m.p. 170–172°C (Lit. m.p.. 170–171°C).[3]

*3-Acetyl-4-benzoyl-1-(4–Chlorophenyl)-5-phenyl-1*H*-pyrazole* (**5d**): This compound was obtained as orange crystals (yield 80%), m.p. 166–167°C. IR (KBr) ν 1684 1663 (2 C=O); ^{1}H NMR (DMSO-d_6): δ 2.60 (s, 3H, $COCH_3$), 7.23–7.84 (m, 14H, ArH); MS: *m/z* (%): 402 (M^++2, 10), 401 (M^++1, 11), 400 (M^+, 30), 385 (15), 323 (45), 111 (17), 105 (44), 77 (100). Anal. Calcd for $C_{24}H_{17}ClN_2O_2$ (400.87): C, 71.91; H, 4.27; N, 6.99. Found: C, 72.15; H, 4.15; N, 6.95%.

3-*Acetyl-4-benzoyl-1-(4-nitrophenyl)-5-phenyl-1*H*-pyrazole* (**5e**): This compound was obtained as pale brown crystals (yield 75%), m.p. 214°C (AcOH); IR (KBr) ν_{max} / cm-1 1686, 1654 (2 C=O); ^{1}H NMR (DMSO-d_6): δ 2.61 (s, 3H, $COCH_3$), 7.27–7.64 (m, 5H, ArH), 7.71 (d, *J* = 9.0 Hz, 2H, ArH), 8.34 (d, *J* = 9.0 Hz, 2H, ArH); MS: *m/z* (%): 412 (M^++1, 10), 411 (M+, 15), 367 (60), 334 (100), 105 (28). Anal. Calcd for $C_{24}H_{17}N_3O_4$ (411.42), C, 70.07; H, 4.16; N, 10.21. Found: C, 70.34; H, 4.27; N, 10.29%.

Synthesis of 3-[(E)-4-benzoyl-3-(N,N-dimethylamino)acryloyl]-1,5-diaryl-1H-pyrazoles **6a–e**
A mixture of the appropriate 3-acetyl-1-aryl-4-benzoyl-5-phenyl-1*H*-pyrazole (**5**) (3.66 g, 10 mmol) and N,N-dimethylformamide-dimethylacetal (DMF-DMA) (2.4 g, 20 mmol) was refluxed for 5 h then left to cool. To the cold mixture was added ether. The resulting yellow solid was collected by filtration, washed with ether, dried and finally crystallised from ethanol to afford the respective enaminone **6**. The various enaminones **3a–e** together with their physical properties are listed below.

*3-[(*E*)-4-benzoyl-3-(*N,N*-dimethylamino)acryloyl]-1-(4-methoxyphenyl)-5-phenyl-1*H*-pyrazole* (**6a**)*:* Yellow crystals in 80 % yield. m.p. 194–196°C (EtOH); IR (KBr) ν_{max}/cm^{-1} 1721, 1689 (2C=O); ^{1}H NMR (DMSO-d_6): δ 2.92 (s, 6H, 2 × CH_3), 3.11 (s, 3H, OCH_3), 5.85 (d, 1H, *J* = 13.0 Hz, –CO–CH=), 6.78 (d, *J* = 9.0 Hz, 2H, ArH), 7.14 (d, *J* = 9.0 Hz, 2H, ArH), 7.25-7.33 (m, 10H ArH), 7.80 (d, 1H, *J* = 13.0 Hz, –CH=N); MS *m/z* (%): 452 (M^++1, 4), 451 (M^+, 6), 434 (14), 412 (11), 346 (24), 105 (49), 77 (100). Anal. Calcd for $C_{28}H_{25}N_3O_3$ (451.53): C, 74.48; H, 5.58; N, 9.31. Found: C, 74.36; H, 5.58; N, 9.41%.

*3-[(*E*)-4-benzoyl-3-(*N,N*-dimethylamino)acryloyl]-1-(4-methylphenyl)-5-phenyl-1*H*-pyrazole* (**6b**)*:* Yellow crystals in 80 % yield. m.p. 184°C (EtOH); IR (KBr) ν_{max}/cm^{-1} 1715, 1649 (2C=O); ^{1}H NMR (DMSO-d_6): δ 2.36 (s, 3H, CH_3), 2.88 (s, 3H, CH_3), 3.12 (s, 3H, CH_3), 5.86 (d, 1H, *J* = 13.0 Hz, –CO–CH=), 7.22–7.62 (m, 14H ArH), 7.69 (d, *J* = 13.0 Hz, 1H, –CH=N); MS m/z (%): 436 (M^++1, 6), 435 (M^+, 13), 418 (22), 330 (42), 218 (12), 98 (95), 77 (100). Anal. Calcd for $C_{28}H_{25}N_3O_2$ (435.53): C, 77.22; H, 5.79; N, 9.65. Found: C, 77.35; H, 5.64; N, 9.64%.

*3-[(*E*)-4-benzoyl-3-(*N,N*-dimethylamino)acryloyl]-1,5-diphenyl-1*H*-pyrazole* (**6c**): Yellow crystals in 70 % yield. m.p. 190–192°C (EtOH) (Lit.[3] 190–192°C).

*3-[(*E*)-4-benzoyl-3-(*N,N*-dimethylamino)acryloyl]-1-(4–Chlorophenyl)-5-phenyl-1*H*-pyrazole* (**6d**)*:* Yellow crystals in 76 % yield. m.p. 180°C (EtOH); IR (KBr) ν_{max}/cm^{-1} 1668, 1642 (2C=O); ^{1}H NMR

(DMSO-d_6): δ 2.91 (s, 3H, CH_3), 3.13 (s, 3H, CH_3), 5.87 (d, J = 13.0 Hz, 1H, –CO–CH=), 7.25 (d, J = 13.0 Hz, 1H, =CH–N–), 7.45–7.78 (m, 10H, ArH); MS *m/z* (%): 456 (M^++1, 2), 455 (M^+, 4), 438 (11), 350 (16), 98 (55), 77 (100). Anal. Calcd for $C_{27}H_{23}ClN_3O_2$ (455.95): C, 71.13; H, 4.86; N, 9.22. Found: C, 71.31; H, 4.81; N, 9.14%.

*3-[(*E*)-4-benzoyl-3-(*N,N*-dimethylamino)acryloyl]-1-(4-nitrophenyl)-5-phenyl-1*H*-pyrazole* (**6e**)*:* This compound was obtained as dark yellow crystals in 85 % yield. m.p. 170°C (AcOH); IR (KBr) ν_{max}/cm^{-1} 1660, 1644 (2C=O); ^{1}H NMR (DMSO-d_6): δ 2.88 (s, 3H, CH_3), 3.12 (s, 3H, CH_3), 5.86 (d, J = 13.0 Hz, 1H, –CO–CH=), 7.22 (d, J = 13.0 Hz, 1H, =CH–N–), 7.46-7.77 (m, 14H, ArH); MS *m/z* (%): 467 (M^++1, 3), 466 (M^+, 8), 449 (14), 361 (16), 98 (100), 77 (83). Anal. Calcd for $C_{27}H_{22}N_3O_3$ (466.52): C, 69.52; H, 4.75; N, 12.01. Found: C, 69.42; H, 4.80; N, 12.22%.

*4-Benzoyl-1,5-diphenyl-3-(1*H*-pyrazol-3-yl)-1*H*-pyrazole* (**7**): A mixture of enaminone (**1**) (0.42 g, 1 mmol) and hydrazine hydrate (1 mL, 99%) in absolute ethanol (30 mL) was refluxed for 2 h, then left to cool. The precipitated product was collected by filtration, washed with ethanol, dried and crystallised from ethanol to afford the pyrazole derivative **7** as white powder in 70 % yield. m.p. 120–122°C; IR (KBr) ν_{max}/cm^{-1} 3148 (NH), 1664 (C=O); ^{1}H NMR (DMSO-d_6) δ 6.35 (s, 1H, pyrazole), 6.78 (s, 1H, pyrazole), 7.09–7.70 (m, 18H, ArH), 12.9 (s, 1H, NH, D_2O-exchangeable); MS *m/z* (%) 390 (M^+, 80), 389 (100), 77 (45). Anal. Calcd for $C_{25}H_{18}N_4O$ (390.44): C, 76.91; H, 4.65; N, 14.35. Found: C, 77.12; H, 4.78; N, 14.22%.

*2-Amino-4-(4-benzoyl-1,5-diphenyl-1*H*-pyrazol-3-yl)pyrimidine* (**8**): Enaminone **6c** (0.42 g, 1 mmol) was added to an ethanolic sodium ethoxide solution [prepared from sodium metal (0.023 g, 1 mmol) and absolute ethanol (50 mL)] then guanidine hydrochloride (0.1 g, 1 mmol) was added. The reaction mixture was refluxed for 12 h, then left to cool and poured into crushed ice. The precipitated product was collected by filtration, washed with ethanol and dried. Recrystallisation from EtOH–DMF afforded the pyrimidine derivatives **8** as yellow powder in 74 % yield. m.p. 125–127 °C; IR (KBr) ν_{max}/cm^{-1} 3258 (NH_2), 1658 (C=O); ^{1}H NMR (DMSO-d_6) δ 6.62 (s, 2H, NH_2, D_2O-exchangeable), 7.0 (s, 1H, C-5 pyrimidine), 7.1–7.72 (m, 18H, ArH), 8.35 (s, 1H, C-6 pyrimidine); MS *m/z* (%) 418 (M^++1, 8.5), 417 (M^+, 18), 399 (25), 77 (100). Anal. Calcd for $C_{26}H_{19}N_5O$ (417.46): C, 74.80; H, 4.59; N, 16.78. Found: C, C, 74.68; H, 4.82; N, 16.75%.

Reaction of enaminone **6c** *with heterocyclic amines:* A mixture of the enaminone **6c** (0.42 g, 1 mmol) and 3-amino-1,2,4-triazole (1 mmol) in absolute ethanol (50 mL) containing piperidine (0.5 mL) was refluxed for 12 h, then left to cool. The solid product was filtered off, washed with ethanol, dried and finally recrystallised from EtOH-DMF to afford the corresponding triazolo[1,5-*a*]pyrimidine **9**. When the above procedure was repeated using 2-aminobenzimidazole, in place of 3-amino-1,2,4-triazole, the pyrimido[1,2-*a*]benzimidazole derivative **10** was obtained.

*5-(4-Benzoyl-1,5-diphenyl-1*H*-pyrazole-3-yl)-1,2,4-triazolo[1,5-a]pyrimidine* (**9**): Yield 75%, m.p. 110–112°C; IR (KBr) ν_{max}/cm^{-1} 1648 (C=O); ^{1}H NMR (DMSO-d_6) δ 7.14–7.72 (m, 15 H, ArH), 7.58 (d, J = 4.5 Hz, 1H, H-6), 8.46 (s, 1H, H-2), 8.84 (d, J = 4.5 Hz, 1H, H-7); MS *m/z* (%) 443 (M^++1, 15), 442 (M^+, 16), 180 (9), 110 (31), 77 (41). Anal. Calcd for $C_{27}H_{18}N_6O$ (442.47): C, 73.29; H, 4.10; N, 18.99. Found: C, 73.44; H, 4.32; N, 19.21%.

*4-(4-Benzoyl-1,5-diphenyl-1*H*-pyrazole-3-yl)pyrimido[1,2-a]-benzimidazole* (**10**): Yield 72%, m.p. 115–117°C; IR (KBr) ν_{max}/cm^{-1} 1643 (C=O); ^{1}H NMR (DMSO-d_6) δ 7.14–7.72 (m, 19 H, ArH), 7.59 (d, J = 4.5 Hz, 1H, H-3), 8.26 (d, J = 4.5 Hz, 1H, H-2); MS *m/z* (%) 492 (M^++1, 23), 491 (M^+, 14), 405 (42), 316 (54), 180 (17), 77 (100). Anal. Calcd for $C_{32}H_{21}N_5O$ (491.54): C, 78.19; H, 4.31; N, 14.2. Found: C, 78.03; H, 4.51; N, 14.28%.

*Synthesis of 3-substituted-6-(4-benzoyl-1,5-diphenylpyrazol-3-yl)-pyridin-2(1H)-ones (***12a,b***)*

To a mixture of the enaminone **6c** and malononitrile (1 mmol of each) in absolute ethanol (25 mL), piperidine (0.5 mL) was added and the reaction mixture was refluxed for 12 h, then left to cool. The solid product was filtered off, washed with ethanol, dried and finally recrystallised from EtOH-DMF to afford the corresponding pyridin-2(1*H*)-one derivative **12a**.

When the above procedure was repeated using ethyl cyanoacetate in place of malononiytrile, product **12b** was obtained.

*6-(4-Benzoyl-1,5-diphenyl-1*H*-pyrazol-3-yl)-3–cyano-2-oxo-pyridin-2(1*H*)-one* (**12a**)*:* Yield 70%, m.p. 115–117°C; IR (KBr) ν_{max}/cm^{-1} 3190 (NH), 2212 (C≡N), 1662, 1649 (2C=O); ^{1}H NMR (DMSO-d_6) δ 7.14–7.73 (m, 15H, ArH), 8.04 (d, J = 9.0 Hz, 1H, pyridine-H-5), 8.60 (d, J = 9.0 Hz, 1H, pyridine-H-4), 12.3 (s, 1H, NH, D_2O-exchangeable); MS *m/z* (%) 443 (M^+, 65), 442 (100), 77 (7). Anal. Calcd for $C_{28}H_{18}N_4O_2$ (442.47): C, 76.01; H, 4.10; N, 12.66. Found: C, 75.86; H, 4.33; N, 12.47%.

*6-(4-Benzoyl-1,5-diphenyl-1*H*-pyrazol-3-yl)-3-ethoxycarbonylpyridin-2(1*H*)-one* (**12b**)*:* Yield 68%, m.p. 107–109°C; IR (KBr) ν_{max}/cm^{-1} 3168 (NH), 1722, 1658, 1618 (3C=O); ^{1}H NMR (DMSO-d_6) δ 1.15 (t, J = 7.0 Hz, 3H, CH_3), 4.14 (q, J = 7.0 Hz, 2H, CH_2), 7.19–7.72 (m, 15 H, ArH), 8.19 (d, J = 9.0 Hz, 1H, pyridine-H-5), 8.60 (d, J = 9 Hz, 1H, pyridine-H-4), 9.32 (s, 1H, NH, D_2O-exchangeable); MS *m/z* (%) 490 (M^++1, 3), 489 (M^+, 10), 444 (9), 400 (37), 180 (24), 105 (100). Anal. Calcd for $C_{30}H_{23}N_3O_4$ (489.52): C, 73.61; H, 4.74; N, 8.58. Found: C, 73.48; H, 4.92; N, 8.35%.

Cytotoxic activity against human breast cancer (MCF-7) in vitro.

The method applied is similar to that reported by Skehan *et al*. using Sulfo-Rhodamine-B stain (SRB).[15] Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the test compound to allow attachment of cell to the wall of the plate. Five different concentrations of the compound **6** under test (0, 5, 12.5, 25, and 50 μg mL^{-1}) 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, the cells were fixed, washed and stained with SRB stain, the excess stain was washed with acetic acid and the attached stain was recovered with tris-EDTA buffer; the colour intensity was measured in an ELISA reader. The relation between the surviving fraction and drug concentration is plotted to obtain the survival curve of tumour cell line and the IC_{50} was calculated. The results are summarised in Table 1.

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