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Synthesis and anticonvulsant activity of some new pyrazolo[3,4-*b*]pyrazines and related heterocycles

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ABSTRACT

A series of new pyrazolo[3,4-*b*]pyrazines containing, 1,2,4-oxadiazolyl, thiadiazolyl, imidazothiadiazolyl, thiazolidinonyl, substituents and other different substituents, was synthesized using 1,6-diphenyl-3-methyl-l*H*-pyrazolo[3,4-*b*]pyrazine-5-carbonitrile (**2**) as a starting material. Some of the newly prepared compounds were evaluated for their anticonvulsant activity. Compounds **9a**, **13a–d** and **14a** at a dose of 10 mg/kg showed very significant anticonvulsant activity and increased the latency time of PTZ-induced tonic seizures. Compound **9b** showed significant effect.

1. Introduction

Pyrazolo[3,4-*b*]pyrazine ring system is an interesting class of heterocycles of great medical importance. It has been reported that some of its derivatives are used as inhibitors of protein kinases,[1] blood platelet aggregation,[2] bone metabolism improvers,[3] antiinflammatories,[2] antifungal,[4,5] antibacterial,[4,6] antiparasitic,[5] and anticancer agents.[7,8] On the other hand, pyrazolo[3,4-*b*]pyrazines are also used as fluorescent[9] and disperse dyes in dye chemistry.[10] Certain derivatives were reported to possess antiviral, antineoplastic and anti-fungal activities.[11–13] Also pyrazolopyrazines were used in the treatment of hematologic diseases,[14] and reported as adenosine antagonists.[15,16] Recently, a microwave-assisted synthesis of fused pyrazolo[3,4-*b*]pyrazines by the reaction of ortho-aminonitrosopyrazoles and cyclic β-diketones was reported.[17] In continuation to our work directed toward the synthesis of new fused pyrazines[5,13,18–20] and pyrazole-based hetrocycles[21] we report herein the synthesis of new pyrazolo[3,4-*b*]pyrazines and related heterocycles. Some derivatives of the compounds prepared were tested for their anticonvulsant activity.

2. Results and discussion

2.1. Chemistry

The starting material 1,6-diphenyl-3-methyl-l*H*-pyrazolo[3,4-*b*]pyrazine-5-carbonitrile (**2**) was prepared via the reaction of the readily available 5-amino-3-methyl-4-nitroso-l-phenylpyrazole (**1**) with benzoyl acetonitrile in refluxing pyridine according to our published procedure[13] (Scheme 1).

When the cyano function of **2** was interacted with hydroxylamine hydrochloride, the product obtained was the amidoxime **3**. Heating this latter compound with benzoylchloride or 4-chlorobenzoyl chloride in refluxing pyridine afforded the corresponding 1,2,4-oxadiazolyl derivatives **4a**,**b**. The IR spectrum of compound **3** showed υ NH_2 and OH bands at $\bar{\nu} = 3450$, 3350 and 3200 cm^{-1}. These absorption bands disappeared in the IR spectra of compounds **4a**,**b**. Also, the ^{1}H NMR spectrum of compound **3** showed a singlet at δ 6.04 ppm (NH_2) and another singlet at δ 9.55 ppm (OH). These two signals disappeared in the ^{1}H NMR spectra of compounds **4a**,**b** (Scheme 2).

The alkaline hydrolysis of **2** gave the carboxylic acid **5** which was esterified in refluxing absolute methanol in the presence of concentrated sulfuric acid to give the corresponding methyl ester **6**. The ester **6** was readily converted into the corresponding acid hydrazide**7**[13] upon heating under reflux with ethanolic solution of hydrazine hydrate. The reaction of **5** with thiosemicarbazide in

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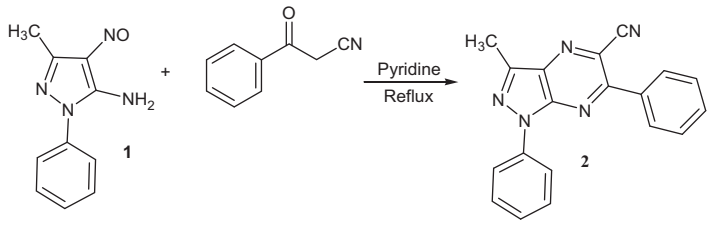
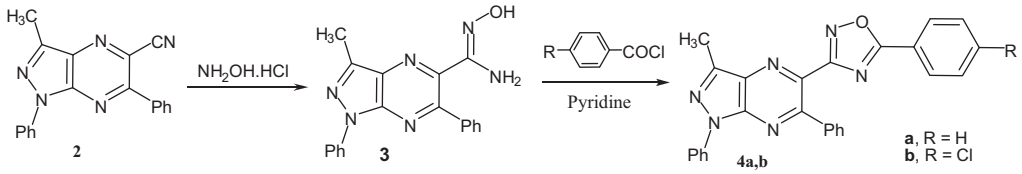


Scheme 1. Synthetic pathway of compound **2**.



Scheme2. Synthetic pathway of compounds **3** and **4a**,**b**.

refluxing phosphorylchloride gave the corresponding amino-thiadiazolyl derivative **8** (Scheme 3).

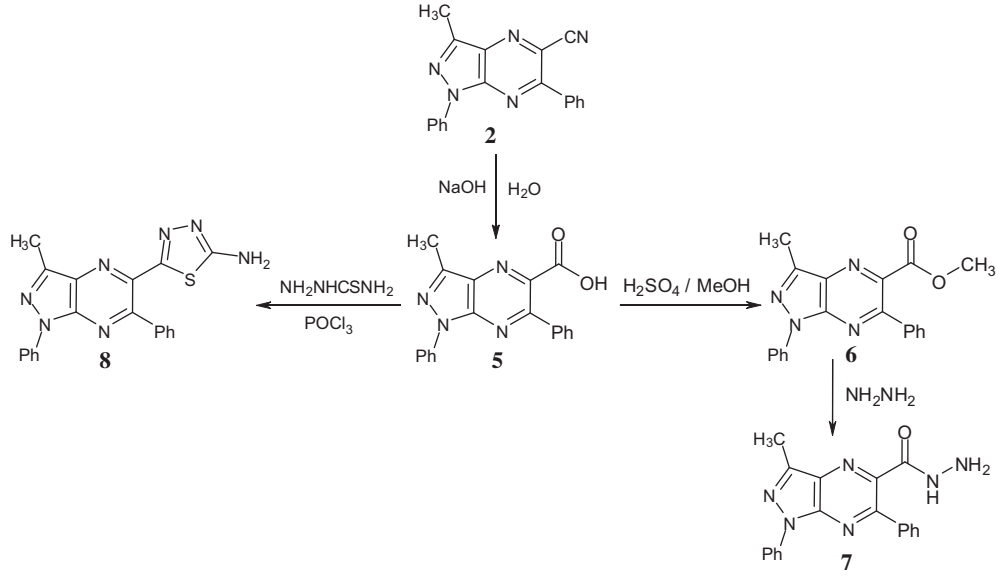
Condensation of **8** with phenacylbromide and 4-bromo-phenacylbromide yielded the corresponding imidazothiadiazolyl derivatives **9a**,**b** (Scheme 4).

Treatment of the carbohydrazide derivative **7** with some aromatic aldehydes, cyclic ketones and acetone gave the corresponding arylidene **10a**–**d**, cycloalkylidene **11a**,**b** and isopropylidene **12** derivatives respectively (Scheme 5). The IR spectra of these compounds showed υ NH bands within $\bar{\nu} = 3300\text{-}3210\ cm^{-1}$ in addition to υ C=O bands around $\bar{\nu} = 1690\text{-}1650\ cm^{-1}$ while their ^1H NMR spectra showed characteristic signals assigned to CH_3, NH, and CH=N protons.

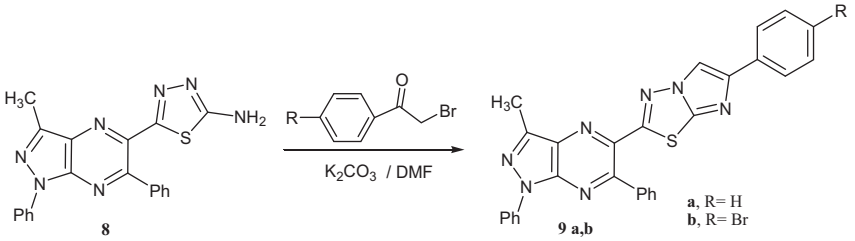
The reaction of the arylidene compounds **10a**–**d** with thioglycolic acid in boiling dry benzene, using a Dean–Stark apparatus gave the corresponding *N*-(2-arylthiazolidin-4-on-3-yl)-1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carboxamide (**13a**–**d**) (Scheme 6).The IR spectra of the latter compounds showed υ NH absorption bands around $\bar{\nu} = 3300$ and υ C=O bands around $\bar{\nu} = 1690\ cm^{-1}$ in addition to the endocyclic υ C=O band of the thiazolidinone ring around $\bar{\nu} = 1720\ cm^{-1}$. Their ^1H NMR showed the characteristic two doublets of the CH_2 protons of the thiazolidinone ring around δ 3.75 ppm (J = 15.6 Hz) and 3.84 ppm (J = 15.6 Hz), a singlet corresponding to the proton at position 2 of the thiazolidinone ring within 5.48–6.09 ppm and the singlet of the NH proton within δ 9.17–9.01 ppm. ^{13}C NMR spectrum of compound **13a** showed new signals at δ 11.36 ppm (CH_3), δ 30.09 ppm (CH_2 thiazolidinone), δ 62.80 ppm (CH thiazolidinone) and δ 169.68 ppm (CO thiazolidinone).

On the other hand, when the above reaction was repeated using the cycloalkylidenes **11a**,**b** or isopropylidene derivative **12**, the spirothiazolidinones **14a**,**b** and **15** were formed as shown in Scheme 7. ^1H NMR spectra of compounds **14a**,**b** and **15** showed disappearance of a singlet corresponding to the proton at position 2 of the thiazolidinone ring and appearance new signals of aliphatic protons.

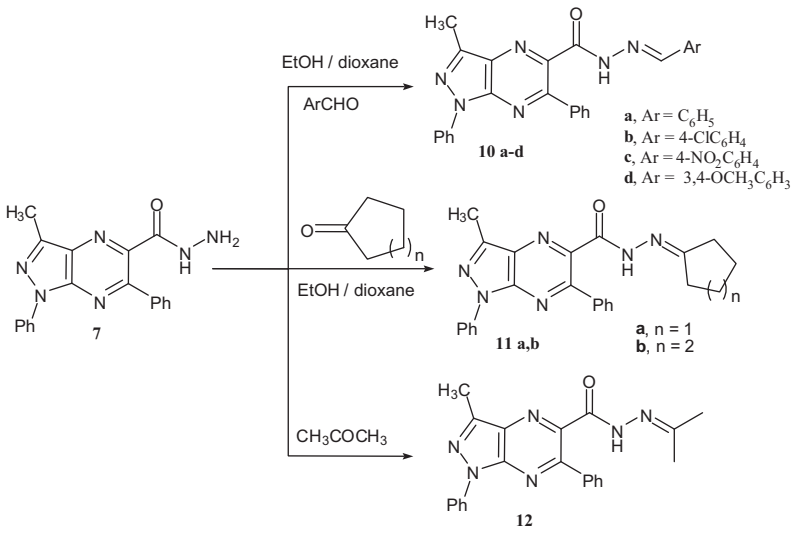
Treatment of carbohydrazide**7** with benzoyl chloride gave *N*′-benzoyl-1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carbohydrazide (**16**) which underwent cyclodehydration when treated with phosphorylchloride giving the oxadiazolyl drerivative **17**. The latter compound **17** could be synthesized unequivocally



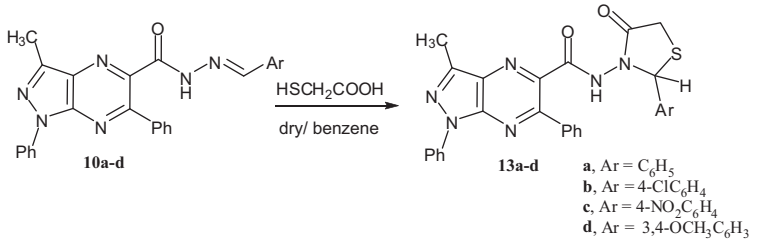
Scheme 3. Synthetic pathway of compounds **5**–**8**.



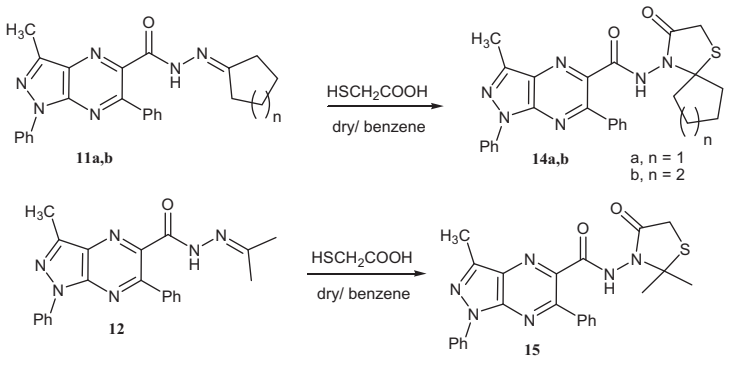
Scheme 4. Synthetic pathway of compounds **9a**,**b**.



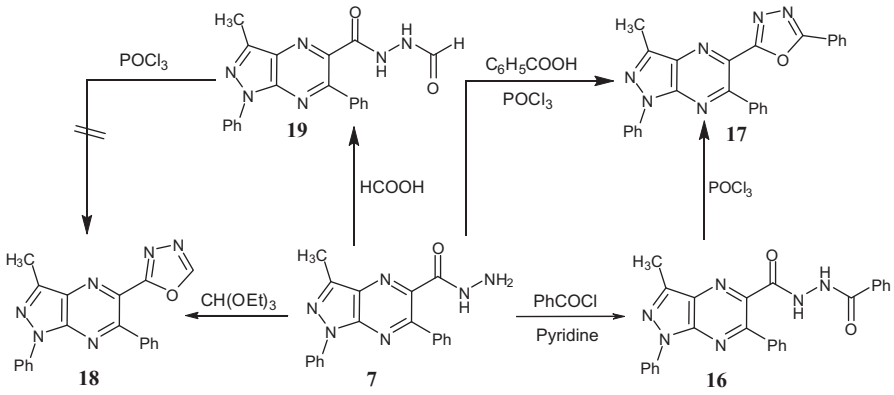
Scheme 5. Synthetic pathway of compounds **10**–**12**.



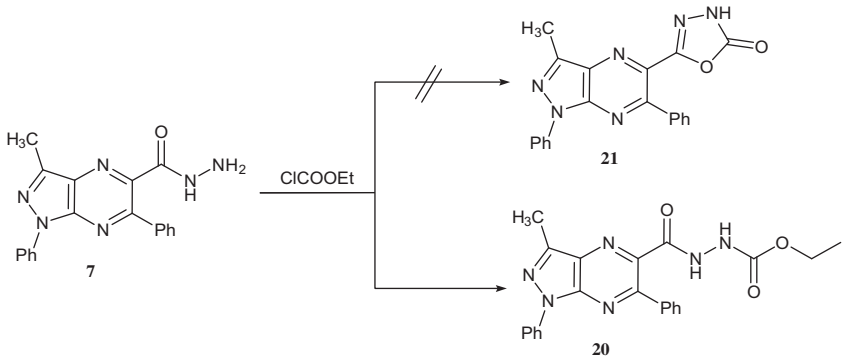
Scheme 6. Synthetic pathway of compounds **13a**–**d**.



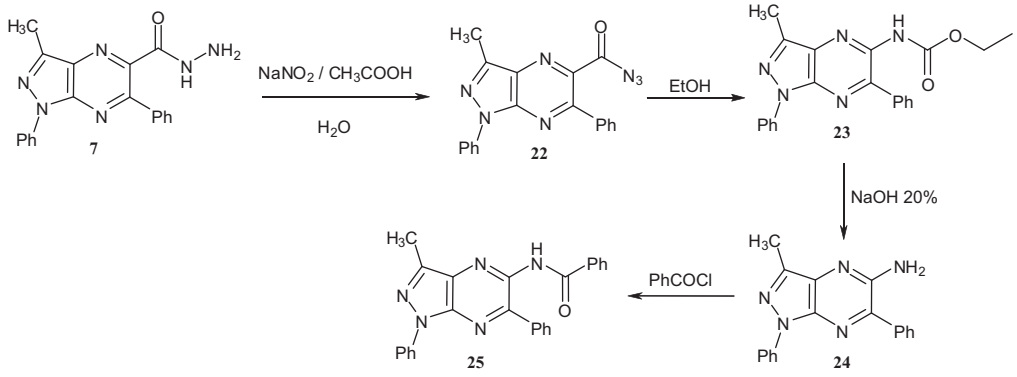
Scheme 7. Synthetic pathway of compounds **14**,**15**.



Scheme 8. Synthetic pathway of compounds **16**–**19**.



Scheme 9. Synthetic pathway of compounds **20**,**21**.



Scheme 10. Synthetic pathway of compounds **22**–**25**.

when the carbohydrazide **16** was reacted with benzoic acid in refluxing phosphorylchloride. Treatment of compound **7** with formic acid yielded *N*′-formylcarbohydrazide derivative **19** which failed to cyclize into the oxadiazole **18** upon boiling in phosphorylchloride. However, **18** could be obtained when **7** was allowed to react with triethylorthoformate (Scheme 8).

When compound **7** was reacted with ethyl chloroformate whether neat or in refluxing ethanol (for 1 h or 24 h), the product obtained was identified as the ethoxycarbonyl derivative **20** and not the expected oxadiazolonyl derivative **21** (Scheme 9).

On the other hand, the substitution of the acid hydrazide function of **7** by an amino group could be performed by converting **7** into the corresponding acid azide **22** under diazotization conditions, followed by heating the azide **22** in refluxing ethanol, followed by alkaline hydrolysis of the produced carbamate **23** to give the amino derivative **24**. The reaction of the latter compound with benzoylchloride gave the benzoylamino derivative **25** (Scheme 10).

2.2. Anticonvulsant activity

Certain derivatives of imidazothiadiazoles and thiazolidinones were reported to possess anticonvulsant activity.[22,23] Accordingly, it deemed of interest to evaluate the anticonvulsant activity of the imidazothiadiazole- and thiazolidinone derivatives of our prepared compounds. The preliminary results of anticonvulsant testing of the compounds listed in the Table 1 revealed that, pretreatment of mice with compounds **9a**,**b**, **13a**–**d** and **14a** at a

Table 1
Anticonvulsant activity[+] (effect of compounds **9a–b**, **13a–d**, **14a**, **b** and **15** on PTZ-induced seizures and mortality in mice)

Treatment	Total number of animals	Clonic convulsions		Tonic convulsions		Survivors (24 h)	% Survivors (24 h)
		Onset (min)	% Animals showing convulsions	Onset (min)	% Animals showing convulsions		
PTZ	8	4.62 ± 0.30	87.5	10.00 ± 0.60	50	4	50
Phenobarbital	8	0	0	0	0	8	100
Comp. **13d** + PTZ	8	4.50 ± 0.38	87.5	0	0	8	100[**]
Comp. **13a** + PTZ	8	4.28 ± 0.35	75	15.90[**] ± 0.51	12.5[**]	7	87.5[**]
Comp. **9a** + PTZ	8	4.85 ± 0.29	87.5	14.61[**] ± 0.72	12.5[**]	7	87.5[**]
Comp. **13c** + PTZ	8	5.25 ± 0.42	87.5	16.21[**] ± 0.92	25[**]	6	75[**]
Comp. **13b** + PTZ	8	4.83 ± 0.32	75	16.12[**] ± 0.88	25[**]	6	75[**]
Comp. **14a** + PTZ	8	5.28 ± 0.41	100	16.32[**] ± 1.10	25[**]	6	75[**]
Comp. **9b** + PTZ	8	5.12 ± 0.41	87.5	18.00[**] ± 1.20	37.5[*]	5	62.5[*]
Comp. **15** + PTZ	8	4.25 ± 0.38	100	9.12 ± 0.72	75	2	25
Comp. **14b** + PTZ	8	4.12 ± 0.36	100	8.56 ± 0.62	100	0	0

The compounds under investigation were injected ip 20 min before ip injection of PTZ.
- PTZ (Pentylenetetrazole).
[+] Compounds prepared were administered at a dose of 10 mg/kg.
[*] Significantly compared with control (p <0.05 vs PTZ value).
[**] Very significantly compared with control (P <0.01 vs PTZ value).

dose of 10 mg/kg, ip significantly increased the latency time of PTZ-induced tonic seizures and decreased the number of animals showing tonic seizures in response to PTZ injection. Also, these results indicated that pretreatment of mice with these compounds significantly inhibited PTZ-induced mortality within 24 h. However, compounds **15** and **14b** increased PTZ-induced tonic convulsions and mortality. All tested compounds showed insignificant effect on PTZ-induced clonic convulsions. Phenobarbital completely prevented PTZ-induced convulsive seizures and mortality.

2.3. Structure–activity relationship

Concerning the anticonvulsant activity against PTZ-induced tonic seizures, compound **13a**, in which the 2-phenyl group of the thiazolidinone moiety is unsubstituted, showed very significant protective activity. The introduction of two methoxyl groups in that phenyl ring (compound **13d**) rendered the compound having the highest activity. The introduction of Cl or NO_2 group in the 4-position of the phenyl group (**13b**,**c**) lowers the activity. Contrarily to compound **13d** having the best activity, compound (**14b**) which has two methyl substituents in the 2-position of the thiazolidinone moiety caused 100% convulsion and 100% mortality. The substitution of the two methyl groups of **14b** by a spiro cyclohexane ring (compound **15**) also enhanced the convulsions and mortality but with a lesser extent that **14b**. However, when the cyclohexane ring was substituted by a cyclopentane one (compound **14a**) an improvement of activity was observed, comparable to that of **13b**,**c**.

On the other hand, 1,6-diphenyl-3-methyl-5-(6-phenylimidazo[2,1-*b*]-1,3,4-thiadiazol-2-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine (**9a**) showed very significant activity comparable to **13a**. The introduction of Cl atom at the 4-position of the 6-phenyl group (**9b**) lowers the acivity.

3. Conclusion

In conclusion, we have made available a series of new pyrazolo[3,4-*b*]pyrazinescontaining heterocyclic substituents, and their anticonvulsant activity was evaluated. The anticonvulsant activity against PTZ-induced convulsions in mice at a dose of 10 mg/kg for each compound showed that seven compounds (**9a**,**b**, **13a–d** and **14a**) showed very significant anticonvulsant activity (p <0.01) with respect to the control (see Table 1) with delayed the onset time of PTZ-induced tonic seizer. Compound **13d** showed best results while compounds **14d** and **15**increased PTZ-induced tonic convulsions and mortality.

4. Experimental section

4.1. Chemistry

All melting points were measured on Stuart melting point apparatus (Bibby Scientific) SMP3. The IR spectra were recorded on a Shimadzu 470 IR-Spectrophotometer using KBr wafer technique. The ^{1}H NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer (Varian, Palo Alto, CA, USA, 300 MHz for ^{1}H and 75 MHz for ^{13}C) at the Faculty of Science, Cairo University, and on a Jeol LA 400 MHz (400 MHz for ^{1}H, 100 MHz for the ^{13}C) at Assiut university, ^{1}H and ^{13}C NMR chemical shifts (δ) were reported in parts per million (ppm) and were referenced to the solvent peak; $CDCl_3$ (7.26 ppm for ^{1}H and 76.90 ppm for ^{13}C) and DMSO-d_6 (2.50 ppm for ^{1}H and 39.70 ppm for ^{13}C). Multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Coupling constants (J) are reported in Hertz (Hz). Elemental analyses were carried out using a Perkin–Elmer 240C Microanalyzer at the Microanalytical Laboratory at Assiut University, and the results obtained were in an acceptable range (±0.4%). 1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carbonitrile (**2**), 1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carboxylic acid (**5**), methyl 1,6-diphenyl-3-methyl-1*H*-pyrazolo [3,4-*b*]pyrazine-5-carboxylate (**6**), 1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-5-carbohydrazide (**7**), 1,6-diphenyl-3-methyl-l*H*-pyrazolo[3,4-*b*]pyrazin-5-carboxylic acid azide (**22**) and ethyl *N*-(1,6-diphenyl-3-methyl-l*H*-pyrazolo[3,4-*b*]pyrazin-5-yl) carbamate (**23**) were prepared in our earlier publication.[13]

4.1.1. 1,6-Diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carboxamidoxime (3)

To a stirred mixture of 1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carbonitrile (**2**) (0.31 g, 1 mmol), and potassium carbonate (0.7 g, 5 mmol) in dioxane (10 mL), was added hydroxylamine hydrochloride (0.35 g, 5 mmol) in water (3.5 mL). The reaction mixture was heated at reflux for 3 h, then suspended in water (5 mL) and stirred for additional 1 h. The solid precipitate obtained was filtered off, dried and recrystallized from ethanol/dioxanemixture (1:1). Yield: 74%; mp: 226–228 °C; IR (ν, cm^{-1}): 3450, 3350, 3200 broad (NH, OH), 1650 (C=N);^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.71 (s, 3H, CH_3), 6.04 (s, 2H, NH_2 exchangeable with

D_2O), 7.35–7.37 (m, 1H, ArH), 7.47–7.61 (m, 5H, ArH), 7.86–7.89 (m, 2H, ArH), 8.24–8.27 (m, 2H, ArH), 9.55 (s, 1H, OH exchangeable with D_2O); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 11.48 (CH_3), 119.96 (C), 126.28 (CH), 128.39 (C), 128.44 (CH), 129.34 (2CH), 129.67 (2CH), 129.19 (2CH), 131.56 (2CH), 138.64 (C), 142.40 (C), 143.38 (C), 143.57 (C), 150.48 (C), 151.98 (C); Anal. Calcd for $C_{19}H_{16}N_6O$ (344.37): C, 66.27; H, 4.68; N, 24.40. Found: C, 66.34; H, 4.60; N, 24.33.

4.1.2. General procedure for the synthesis of 5-(aryl-1,2,4-oxadiazol-3-yl)-1,6-diaryl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine 4a,b

Benzoyl chloride derivative (1 mmol) was added to a stirred solution of **3** (0.34 g, 1 mmol) in pyridine (5 mL). The reaction mixture was heated under reflux for 3 h, cooled and poured onto cold water. The solid precipitate formed was filtered, washed with water, dried and recrystallized from ethanol.

4.1.2.1. 1,6-Diphenyl-3-methyl-5-(5-phenyl-1,2,4-oxadiazol-3-yl) -1H-pyrazolo[3,4-b]pyrazine (4a). Yield: 77%; mp: 204–206 °C; IR (ν, cm^{-1}): 3050 (CH aromatic), 2900 (CH aliphatic); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.75 (s, 3H, CH_3), 7.37–7.38 (m, 1H, ArH), 7.45–7.73 (m,10H, ArH), 8.07–8.09 (d, 2H, ArH), 8.26 - 8.29 (d, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 11.30 (CH_3), 120.19 (C and 2CH), 122.99 (C), 126.24 (CH), 128.01 (2CH), 128.50 (C and CH), 128.70 (CH), 129.49 (2CH), 129.74 (2CH), 132.70 (2CH), 133.82 (2CH), 136.07 (C), 137.37 (C), 138.39 (C), 144.18 (C), 153.20 (C), 168.24 (C), 175.61(C); Anal. Calcd for $C_{26}H_{18}N_6O$ (430.46): C, 72.55; H, 4.21; N, 19.52. Found C, 72.50; H, 4.36; N, 19.49.

4.1.2.2. 5-(5-(4-Chlorophenyl)-1,2,4-oxadiazol-3-yl)-1,6-diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazine (4b). Yield: 79%; mp: 182–184 °C; IR (ν, cm^{-1}): 3050 (CH aromatic), 1590 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.78 (s, 3H, CH_3), 7.24–7.28 (m, 1H, ArH), 7.36 (d, J = 8 Hz, 2H, ArH), 7.44–7.49 (m, 5H, ArH), 7.55–7.57 (m, 2H, ArH), 8.01 (d, J = 8 Hz, 2H, ArH), 8.28 (d, J = 7.6 Hz, 2H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 11.69 (CH_3), 120.24 (C and 2CH), 122.22 (C), 126.23 (CH), 128.40 (C and CH), 129.28 (2CH), 129.57 (2CH), 129.65 (2CH), 133.01 (2CH), 136.35 (2CH), 137.67 (C), 138.87 (C), 139.58 (C), 142.87 (C), 144.46 (C), 153.20 (C), 168.72 (C), 175.18 (C); Anal. Calcd for $C_{26}H_{17}ClN_6O$ (464.91): C, 67.17; H, 3.69; Cl, 7.63; N, 18.08. Found: C, 67.27 ; H, 3.60; Cl, 7.59; N, 18.18.

4.1.3. 5-(5-Amino-1,3,4-thiadiazol-2-yl)-1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (8)

A mixture of the acid 1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*] pyrazine-5-carboxylic acid (**5**)[13] (3 g, 9 mmol), and thiosemicarbazide (8.27 g, 90 mmol) in phosphorylchloride (15 mL) was refluxed gently for 3 h. The reaction mixture was cooled and quenched (highly exothermic) with cold water (27 mL), and the resulting solution was refluxed for additional 4 h. The solution is allowed to cool and basified with aqueous potassium hydroxide solution. The solid that separated out was filtered, washed with water, dried and recrystallized from dioxane/ DMF mixture (3:1). Yield: 77%; mp: 292–294 °C; IR (ν, cm^{-1}): 3350, 3100 (NH_2), 2950, 2900, 2830 (CH aliphatic); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.68 (s, 3H, CH_3), 7.31–7.34 (m, 1H, ArH), 7.44–7.47 (m, 5H, ArH and NH_2), 7.54–7.61 (m, 4H, ArH), 8.19–8.21 (m, 2H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 11.28 (CH_3), 108.09 (C), 119.85 (2CH), 126.23 (CH), 128.02 (CH), 129.03 (C), 129.49 (2CH), 129.60 (2CH), 131.87 (2CH), 138.62 (C), 139.81 (C), 141.61 (C), 143.46 (C), 151.59 (C), 155.80 (C), 170.01 (C); Anal. Calcd for $C_{20}H_{15}N_7S$ (385.45): C, 62.32; H, 3.92; N, 25.44; S, 8.32. Found: C, 62.52; H, 3.73; N, 25.41; S, 8.11.

4.1.4. General procedure for the synthesis of 1,6-diphenyl-3-methyl-5-(6-(substitutedphenyl)imidazo[2,1-*b*]-1,3,4-thiadiazol-2-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine 9a,b

A mixture of equimolar amounts of compound **8** (0.38 g, 1 mmol), phenacylbromide derivative (0.001 mol) and potassium carbonate (0.5 g) was refluxed in DMF (10 mL) for 3 h. The excess of solvent was removed under reduced pressure and the solid obtained was collected by filtration, washed with water, dried and recrystallized from ethanol.

4.1.4.1. 1,6-Diphenyl-3-methyl-5-(6-phenylimidazo[2,1-b]-1,3,4-thiadiazol-2-yl)-1H-pyrazolo[3,4-b]pyrazine (9a). Yield: 46%; mp: 260–262 °C; IR (ν, cm^{-1}): 3130, 3050 (CH aromatic), 1600 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.76 (s, 3H, CH_3), 7.24–7.27 (m, 1H, ArH), 7.36–7.62 (m, 8H, ArH), 7.74 (d, J = 7.2 Hz, 2H, ArH), 7.85 (d, J = 7.6 Hz, 2H, ArH), 8.23 (d, J = 8.4 Hz, 2H, ArH), 8.52 (s, 1H, CH imidazole); Anal.Calcd for $C_{28}H_{19}N_7S$ (485.57): C, 69.26; H, 3.94; N, 20.19; S, 6.60. Found: C, 69.33; H, 3.90; N, 20.28; S, 6.61.

4.1.4.2. 1,6-Diphenyl-3-methyl-5-(6-(4-bromophenyl) imidazo [2,1-b]-1,3,4-thiadiazol-2-yl)-1H-pyrazolo[3,4-b]pyrazine (9b). Yield: 78%, mp: 292–294 °C; IR (ν, cm^{-1}): 3140 (CH aromatic); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.78 (s, 3H, CH_3), 7.38–7.41 (m, 1H, ArH), 7.54 (d, J = 7.5 Hz, 2H, ArH), 7.57–7.64 (m, 5H, ArH), 7.76 (d, J = 6.8 Hz, 2H, ArH), 7.82 (d, J = 7.5 Hz, 2H, ArH), 8.24 (d, J = 8.4 Hz, 2H, ArH), 8.59 (s, 1H, CH imidazole); Anal. Calcd for $C_{28}H_{18}BrN_7S$ (564.47):C, 59.58; H, 3.21; Br, 14.16; N, 17.37; S, 5.68. Found: C, 59.52; H, 3.22; Br, 14.11; N, 17.32; S, 5.71.

4.1.5. General procedure for the synthesis of N′-arylidene-1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carbohydrazide 10a–d

A mixture of equimolar amounts (1 mmol) of 3-methyl-1,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carbohydrazide (**7**)[13] and the appropriate aromatic aldehyde in a mixture of ethanol/dioxane (8:2 mL) was refluxed for 2 h. After cooling, the solid precipitate formed was collected by filtration and recrystallized from ethanol/dioxane mixture (3:1).

4.1.5.1. N′-Benzylidene-1,6-diphenyl-3-methyl-1H-pyrazolo [3,4-b]pyrazine-5-carbohydrazide (10a). Yield: 91%; mp: 248–250 °C; IR (ν, cm^{-1}): 3220 (NH), 3050 (CH aromatic), 2910, 2820 (CH aliphatic), 1680 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.73 (s, 3H, CH_3), 7.34–7.37 (m, 1H, ArH), 7.45–7.83 (m, 10H, ArH), 8.25–8.30 (m, 4H, ArH), 8.35 (s, 1H, CH=N), 12.11 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 11.28 (CH_3), 119.95 (C), 120.01 (2CH), 126.71 (CH), 128.73 (C), 128.90 (CH), 128.98 (2CH), 129.52 (2CH), 129.73 (2CH), 129.80 (2CH), 130.44 (2CH), 131.29 (CH), 137.22 (C), 138.42 (C), 138.59 (C), 143.55 (C), 143.71 (CH), 148.74 (C), 150.19 (C), 168.61 (C=O); Anal. Calcd for$C_{26}H_{20}N_6O$ (432.49):C, 72.21; H, 4.66; N, 19.43. Found: C, 72.33; H, 4.59; N, 19.49.

4.1.5.2. N′-(4-Chlorobenzylidene)-1,6-diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazine-5-carbohydrazide (10b). Yield: 92%; mp: 258–260 °C; IR (ν, cm^{-1}): 3220 (NH), 3060 (CH aromatic), 2910, 2850 (CH aliphatic), 1685 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.73 (s, 3H, CH_3), 7.24–7.79 (m, 10H, ArH), 8.25–8.29 (m, 4H, ArH), 8.34 (s, 1H, CH=N), 12.15 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 11.31 (CH_3), 119.99 (C), 126.26 (2CH), 126.44 (CH), 128.65 (C), 128.39 (CH), 129.54 (2CH), 129.70 (2CH), 129.01 (2CH), 131.40 (2CH), 130.85 (2CH), 137.25 (C), 138.69 (C), 142.47 (C), 143.64 (CH), 147.47 (C), 150.30 (C), 151.31 (C), 162.97 (C), 168.76 (C=O); Anal. Calcd for $C_{26}H_{19}ClN_6O$ (466.92): C, 66.88; H, 4.10; Cl, 7.59; N, 18.00. Found: C, 66.90; H, 4.21; Cl, 7.50; N, 18.11.

4.1.5.3. N′-(4-Nitrobenzylidene)-1,6-diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazine-5-carbo-hydrazide (10c). Yield: 90%; mp: 152–154 °C; IR (*ν*, cm^{-1}): 200 (NH), 3050 (CH aromatic), 2910-2830 (CH aliphatic), 1680 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.74 (s, 3H, CH_3), 7.35–7.39 (m, 1H, ArH), 7.45–7.82 (m, 5H, ArH), 7.99 (d, *J* = 8.4 Hz, 2H, ArH), 8.12 (d, *J* = 8.8 Hz, 2H, ArH) 8.25–8.31 (m, 4H, ArH),8.47 (s, 1H, CH=N), 12.39 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 11.33 (CH_3), 120.06 (C), 123.99 (2CH), 124.12 (2CH), 126.18 (CH), 127.69 (2CH), 128.32 (C), 128.67 (CH), 129.52 (2CH), 129.78 (2CH), 139.53 (C),130.79 (2CH), 138.64 (C), 139.52 (C), 146.47 (C), 147.70 (CH), 148.10 (C), 150.32 (C), 151.34 (C), 162.92 (C), 168.91 (C=O); Anal. Calcd for $C_{26}H_{19}N_7O_3$ (477.47): C, 65.40; H, 4.01; N, 20.53. Found:C, 65.51; H, 3.91; N, 20.49.

4.1.5.4. N′-(3,4-Dimethoxybenzylidene)-1,6-diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazine-5-carbohydrazide (10d). Yield: 72%; mp: 230–232 °C; IR (*ν*, cm^{-1}): 3210 (NH), 3050 (CH aromatic), 2920-2810 (CH aliphatic), 1650 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6):δ 2.73 (s, 3H, CH_3), 3.67 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 6.81–7.22 (m, 3H, ArH), 7.33–7.38 (m, 1H, ArH), 7.47–7.65 (m, 5H,ArH), 7.79–7.83 (m, 2H, ArH), 8.25–8.27 (m, 2H, ArH), 8.28 (s, 1H, CH=N), 12.19 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 11.28 (CH_3), 55.46 (OCH_3), 55.59 (OCH_3), 108.10 (C), 108.25 (CH), 111.44 (CH), 119.77 (2CH), 122.26 (CH), 126.35 (CH), 128.63 (C), 128.96 (CH), 129.52 (2CH), 129.70 (2CH), 130.80 (2CH), 131.20 (C), 137.22 (C), 138.57 (C), 143.49 (C), 148.56 (C), 149.05 (C), 150.48 (C), 150.99 (C), 151.19 (C), 168.45 (C=O); Anal. Calcd for $C_{28}H_{24}N_6O_3$ (492.53): C, 68.28; H, 4.91; N, 17.06. Found:C, 68.20; H, 4.98; N, 17.18.

4.1.6. General procedure for the synthesis of N′-cychloalky lidene-1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carbohydrazide 11a,b

A mixture of equimolar amounts (1 mmol) of the carbohydrazide**7** and the appropriate cycloalkanone (cyclopentanone or cyclohexanone) in a mixture of ethanol/dioxane (8:2 mL) was refluxed for 2 h. After cooling, the solid precipitate obtained was collected by filtration and recrystallized from ethanol.

4.1.6.1. N′-Cyclopentylidene-1,6-diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazine-5-carbohydrazide (11a). Yield: 66%; mp: 216–218 °C; IR (*ν*, cm^{-1}): 3300 (NH), 3050 (CH aromatic), 2970, 2890 (CH aliphatic), 1690 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.56–2.02 (m, 4H, $2CH_2$), 2.34–2.61 (m, 4H, $2CH_2$), 2.77 (s, 3H, CH_3), 7.26–7.31 (m, 1H, ArH), 7.46–7.54 (m, 5H, ArH), 7.76–7.82 (m, 2H, ArH), 8.29–8.35 (m, 2H, ArH), 9.63 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 11.63 (CH_3), 24.81 (CH_2), 27.54 (CH_2), 33.61 (CH_2), 38.41 (CH_2), 120.11 (C and 2CH), 126.23 (CH), 128.27 (C), 128.16 (CH), 129.55 (2CH), 129.26 (2CH), 130.10 (2CH), 138.79 (C), 137.81 (C), 144.00 (C), 153.56 (C), 160.90 (C), 165.38 (C=O), 168.81 (N=C); Anal. Calcd for $C_{24}H_{22}N_6O$ (410.47):C, 70.23; H, 5.40; N, 20.47. Found: C, 70.36; H, 5.49; N, 20.25.

4.1.6.2. N′-Cyclohexylidene-1,6-diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazine-5-carbohydrazide (11b). Yield: 88%; mp: 218–219 °C; IR (*ν*, cm^{-1}): = 3300 (NH), 3050 (CH aromatic), 2910, 2820 (CH aliphatic), 1670 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 1.38–1.70 (m, 6H, $3CH_2$), 2.18–2.25 (m, 4H, $2CH_2$), 2.72 (s, 3H, CH_3), 7.36–7.37 (m, 1H, ArH), 7.51–7.63 (m, 5H, ArH), 7.74–7.83 (m, 2H, ArH), 8.25–8.28 (m, 2H, ArH), 10.85 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 11.29 (CH_3), 24.97 (CH_2), 26.66 (CH_2), 26.88 (CH_2), 27.43 (CH_2), 34.63 (CH_2), 119.85 (C), 119.91 (2CH), 126.23 (CH), 128.71 (C), 128.51 (CH), 129.63 (2CH), 129.51 (2CH), 130.89 (2CH), 138.56 (C), 146.25 (C), 150.77 (C), 158.68 (C), 162.70 (C), 164.61 (C=O), 168.68 (N=C); Anal. Calcd for $C_{25}H_{24}N_6O$ (424.5): C, 70.73; H, 5.70; N, 19.80. Found; C, 70.92; H, 5.72; N, 19.71.

4.1.7. 1,6-Diphenyl-3-methyl-N′-(prop-2-ylidene)-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carbohydrazide (12)

A solution of carbohydrazide**7** (0.34 g, 1 m mol) in acetone (10 mL) was refluxed for 1 h. After cooling, the solid precipitate obtained was collected by filtration and recrystallized from ethanol. Yield: 82%; mp: 220–222 °C; IR(*ν*, cm^{-1}): 3300 (NH), 3050 (CH aromatic), 2910, 2820 (CH aliphatic), 1670 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.98 (s, 3H, N=CCH_3), 2.16 (s, 3H, N=CCH_3), 2.77 (s, 3H, CH_3), 7.29–7.32 (m, 1H, ArH), 7.46–7.82 (m, 5H, ArH), 8.30 (d, *J* = 7.8 Hz, 2H, ArH), 8.35 (d, *J* = 7.8 Hz, 2H, ArH), 9.88 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 11.63 (CH_3), 16.79 (CH_3), 25.61 (CH_3), 120.08 (C and 2CH), 126.21 (CH), 128.30 (C), 128.11 (CH), 129.52 (2CH), 129.24 (2CH), 131.15 (2CH), 138.75 (C), 137.83 (C), 143.92 (C), 143.15 (C), 153.74 (C), 156.79 (C=O), 160.88 (N=C); Anal. Calcd for $C_{22}H_{20}N_6O$ (384.43): C, 68.73; H, 5.24; N, 21.86. Found: C, 68.65; H, 5.29; N, 21.79.

4.1.8. General procedure for the synthesis of *N*-(2-aryl-4-oxo-1,3-thiazolidin-3-yl)-1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carboxamide 13a–d

A mixture of compounds **10a**–**d** (1 mmol) and mercaptoacetic acid (0.18 g, 2 mmol) in dry benzene (50 mL) was refluxed in Dean–stark apparatus for (24 h for **13b**,**d**, 48 h for **13a**,**c**). The solvent was evaporated and the residue obtained was triturated with 10% sodium bicarbonate solution. The precipitate formed was filtered off, washed with water, air dried and recrystallized from ethanol.

4.1.8.1. N-(2-Phenyl-4-oxo-1,3-thiazolidin-3-yl)-1,6-diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazine-5-carboxamide (13a). Yield: 89%; mp: 206–208 °C; IR (*ν*, cm^{-1}): 3230 (NH), 3050 (CH aromatic), 2980, 2910 (CH aliphatic), 1720 (C=O thiazolidinone), 1680 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.64 (s, 3H, CH_3), 3.75 (d, *J* = 15.6 Hz, 1H, CH_2 thiazolidinone), 3.84 (d, *J* = 15.6 Hz, 1H, CH_2 thiazolidinone), 6.01 (s, 1H, CH thiazolidinone), 7.25–7.29 (m, 1H, ArH), 7.42–7.49 (m, 10H, ArH), 7.61–7.62 (m, 2H, ArH), 8.22–8.24 (d, *J* = 7.8 Hz, 2H, ArH), 9.03 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 11.36 (CH_3), 30.09 (CH_2 thiazolidinone), 62.80 (CH thiazolidinone), 120.09 (2CH and C), 126.31 (CH), 128.99-129.64 (12 CH), 131.12 (C), 137.04 (C), 137.71 (C), 138.55 (C), 142.87 (C), 144.27 (C), 153.96 (C), 163.18 (C=O), 169.68 (C=O thiazolidinone); Anal. Calcd for $C_{28}H_{22}N_6O_2S$ (506.58): C, 66.39; H, 4.38; N, 16.59; S, 6.33. Found: C, 66.42; H, 4.31; N, 16.63; S, 6.23.

4.1.8.2. N-(2-(4-Chlorophenyl)-4-oxo-1,3-thiazolidin-3-yl)-1,6-diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazine-5-carboxamide (13b). Yield: 98%; mp: 200–202 °C; IR (*ν*, cm^{-1}): 3360 (NH), 3070 (CH aromatic), 2950, 2850 (CH aliphatic), 1720 (C=O thiazolidinone), 1690 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.68 (s, 3H, CH_3), 3.75 (d, *J* = 15.6 Hz, 1H, CH_2 thiazolidinone), 3.84 (d, *J* = 15.6 Hz, 1H, CH_2 thiazolidinone), 5.98 (s, 1H, CH thiazolidinone), 7.29–7.31 (m, 1H, ArH), 7.39–7.41 (d, *J* = 8.8 Hz, 2H, ArH), 7.43–7.45 (d, *J* = 8.8 Hz, 2H, ArH), 7.47–7.50 (m, 5H, CH ArH), 7.59 (m, 2H, CH ArH), 8.25 (d, *J* = 7.8 Hz, 2H, CH ArH), 9.03 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 11.41 (CH_3), 29.12 (CH_2 thiazolidinone), 60.76 (CH thiazolidinone), 120.01 (C), 126.38 (CH), 128.45 (CH), 128.65 (C), 129.06 (2CH), 129.50 (2CH), 129.60 (2CH), 129.74 (2CH), 130.77 (4CH), 136.81 (C), 137.47 (C), 138.32 (C), 141.98 (C), 142.40 (C), 143.70 (C), 151.17 (C), 164.98 (C=O), 168.91 (C=O thiazolidinone); Anal. Calcd for $C_{28}H_{21}ClN_6O_2S$ (541.02): C, 62.16; H, 3.91; Cl, 6.55; N, 15.53; S, 5.93. Found: C, 62.22; H, 3.96; Cl, 6.50; N, 15.49; S, 5.90.

4.1.8.3. N-(2-(4-Nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl)-1,6-diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazine-5-carboxamide (13c). Yield: 98%; mp: 234–236 °C; IR (*ν*, cm^{-1}): = 3350 (NH), 3070 (CH aromatic), 2910, 2850 (CH aliphatic), 1720 (C=O thiazolidinone), 1690 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$):*δ* 2.66 (s, 3H, CH_3), 3.79 (d, *J* = 15.6 Hz, 1H, CH_2 thiazolidinone), 3.86 (d, *J* = 15.6 Hz, 1H, CH_2 thiazolidinone), 6.09 (s, 1H, CH thiazolidinone), 7.27–7.31 (m, 1H, ArH), 7.46–7.52 (m, 5H, ArH), 7.56–7.58 (m, 2H, ArH), 7.67–7.69 (m, 2H, ArH), 8.22–8.24 (m, 2H, ArH), 8.26–8.28 (m, 2H, ArH), 9.17 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* (ppm): 11.40 (CH_3), 29.00 (CH_2 thiazolidinone), 60.30 (CH thiazolidinone), 120.01 (2CH and C), 123.84 (2CH), 126.38 (CH), 128.78 (CH and C), 128.99 (2CH), 129.50 (2CH), 129.65 (2CH), 130.82 (2CH), 138.32 (C), 141.89 (C), 142.39 (C), 143.72 (C), 146.22 (C), 147.69 (C), 151.22 (C), 165.09 (C=O), 168.98 (C=O thiazolidinone); Anal. Calcd for $C_{28}H_{21}N_7O_4S$ (551.58): C, 60.97; H, 3.84; N, 17.78; S, 5.81. Found: C, 60.89; H, 3.85; N, 17.84; S, 5.95.

4.1.8.4. N-(2-(3,4-Dimethoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl)-1,6-diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazine-5-carboxamide (13d). Yield: 84%; mp: 212–214 °C; IR (*ν*, cm^{-1}): = 3300 (NH), 3070 (CH aromatic), 2950–2830 (CH aliphatic), 1715 (C=O thiazolidinone), 1680 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$):*δ* 2.67 (s, 3H, CH_3), 3.74 (d, *J* = 15.6 Hz, 1H, CH_2 thiazolidinone), 3.84 (d, *J* = 15.6 Hz, 1H, CH_2 thiazolidinone), 3.89 (s, 6H, $2OCH_3$), 5.97 (s, 1H, CH thiazolidinone), 6.85 (m, 1H, ArH), 6.98–7.01 (m, 1H, ArH), 7.09 (m, 1H, ArH), 7.28–7.31 (m, 1H, ArH), 7.47–7.50 (m, 5H, ArH), 7.60–7.62 (m, 2H, ArH), 8.25 (d, *J* = 7.8 Hz, 2H, ArH), 9.01 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* (ppm): 11.41 (CH_3), 30.22 (CH_2 thiazolidinone), 55.05 (OCH_3), 55.96 (OCH_3), 63.03 (CH thiazolidinone), 110.47 (CH), 110.65 (CH), 120.18 (C), 121.26 (CH), 126.38 (CH), 128.02 (CH), 128.83 (C), 129.24 (2CH), 129.42 (2CH), 129.52 (2CH), 131.15 (2CH), 137.63 (C), 138.57 (C), 142.39 (C), 144.28 (C), 149.51 (2C), 150.16 (C), 154.04 (C), 163.17 (C=O), 168.64 (C=O thiazolidinone); Anal. Calcd for $C_{30}H_{26}N_6O_4S$ (566.63):C, 63.59; H, 4.62; N, 14.83; S, 5.66. Found: C, 63.68; H, 4.59; N, 14.89; S, 5.76.

4.1.9. General procedure for the synthesis of *N*-(cycloalkyl-spiro-2-(4-oxo-1,3-thiazolidin-3-yl))-1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carboxamide 14a,b

These compounds were prepared using the same procedure as described before in synthesis of compounds **13a–d**.

4.1.9.1. N-(Cyclopentane-spiro-2-(4-oxo-1,3-thiazolidin-3-yl))-1,6-diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazine-5-carboxamide (14a). Yield: 85%; mp: 232–234 °C; IR (*ν*, cm^{-1}): 3250 (NH), 3050 (CH aromatic), 2950-2830 (CH aliphatic), 1720 (C=O thiazolidinone), 1680 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* (ppm): 1.79 (m, 4H, $2CH_2$), 2.01–2.05 (m, 2H, CH_2), 2.17–2.24 (m, 2H, CH_2), 2.75 (s, 3H, CH_3), 3.66 (s, 2H, CH_2 thiazolidinone) 7.27–7.31 (m, 1H, ArH), 7.46–7.49 (m, 5H, ArH), 7.71–7.72 (m, 2H, ArH), 8.25–8.27 (m, 2H, ArH), 9.24 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* (ppm): 11.55 (CH_3), 22.64 ($2CH_2$ cyclopentane), 38.63 ($2CH_2$ cyclopentane), 29.40 (CH_2 thiazolidinone), 66.70 (C thiazolidinone), 120.16 (C and 2CH), 126.35 (CH), 127.99 (C and CH), 129.24 (2CH), 129.55 (2CH), 131.07 (2CH), 137.85 (C), 138.60 (C), 142.93 (C), 144.20 (C), 154.20 (C), 164.09 (C=O), 168.60 (C=O thiazolidinone); Anal. Calcd for $C_{26}H_{24}N_6O_2S$ (484.57): C, 64.44; H, 4.99; N, 17.34; S, 6.62. Found: C, 64.56; H, 4.91; N, 17.14; S, 6.72.

4.1.9.2. N-(Cyclohexane-spiro-2-(4-oxo-1,3-thiazolidin-3-yl))-1,6-diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazine-5-carboxamide (14b). Yield: 91.8%; mp: 240–242 °C; IR (*ν*, cm^{-1}): = 3230 (NH), 2920, 2850 (CH aliphatic), 1720 (C=O thiazolidinone), 1680 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) *δ* (ppm): 1.41 (m, 6H, $3CH_2$), 1.53 (m, 2H, CH_2), 1.65–1.73 (m, 2H, CH_2), 2.76 (s, 3H, CH_3), 3.61 (s, 2H, CH_2 thiazolidinone), 7.37–7.40 (m, 1H, ArH), 7.53–7.63 (m, 5H, ArH), 7.83–7.86 (m, 2H, ArH), 8.24–8.27 (d, 2H, ArH), 10.81 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (75 MHz, DMSO-d_6) *δ* (ppm): 11.43 (CH_3), 22.78 ($2CH_2$ cyclohexane), 23.75 (CH_2 cyclohexane), 27.82 ($2CH_2$ cyclohexane), 36.72 (CH_2 thiazolidinone), 72.41 (C thiazolidinone), 119.98 (C and CH), 126.35 (CH), 128.44 (CH), 129.42 (C), 129.50 (2CH), 129.69 (2CH), 131.12 (2CH), 137.14 (C), 138.39 (C), 143.26 (C), 143.75 (C), 151.34 (C), 165.82 (C=O), 167.65 (C=O thiazolidinone); Anal. Calcd for $C_{27}H_{26}N_6O_2S$ (498.6): C, 65.04; H, 5.26; N, 16.86; S, 6.43. Found: C, 65.15; H, 5.14; N, 16.94; S, 6.40.

4.1.10. *N*-(2,2-Dimethyl-4-oxo-1,3-thiazolidin-3-yl)-1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carboxamide (15)

A mixture of compound **12** (0.38 g, 1 mmol) and mercaptoacetic acid (0.18 g, 2 mmol) in dry benzene (50 mL) was refluxed in Dean–stark apparatus for 72 h. The solvent was then evaporated under reduced pressure and the residue obtained was triturated with 10% sodium bicarbonate solution. The precipitate formed was filtered off, washed with water, air dried and recrystallized from ethanol. Yield: 96%; mp: 238–240 °C; IR (*ν*, cm^{-1}): 3200 (NH), 3050 (CH aromatic), 2980–2810 (CH aliphatic), 1725 (C=O thiazolidinone), 1690 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* (ppm): 1.64 (s, 6H, $2CH_3$), 2.70 (s, 3H, CH_3), 3.61 (s, 2H, CH_2 thiazolidinone), 7.22–7.25 (m, 1H, ArH), 7.40–7.45 (m, 5H, ArH), 7.64–7.67 (m, 2H, ArH), 8.22 (d, *J* = 8.8 Hz, 2H, ArH), 9.01 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* (ppm): 11.58 (CH_3), 29.38 ($2CH_3$), 29.60 (CH_2 thiazolidinone), 66.95 (C thiazolidinone), 120.19 (C and 2CH), 126.36 (CH), 128.02 (C and CH), 129.26 (2CH), 129.55 (2CH), 131.15 (2CH), 137.80 (C), 138.65 (C), 143.00 (C), 144.31 (C), 154.38 (C), 164.06 (C=O), 168.11 (C=O thiazolidinone); Anal. Calcd for $C_{24}H_{22}N_6O_2S$ (458.54): C, 62.86; H, 4.84; N, 18.33; S, 6.99. Found: C, 62.80; H, 4.91; N, 18.43; S, 6.90.

4.1.11. *N′*-Benzoyl-1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carbohydrazide (16)

To a solution of carbohydrazide **7** (0.34 g, 1 mmol) in pyridine (10 ml), benzoyl chloride (0.14 g, 1 mmol) was added, and the reaction mixture was stirred at room temperature for 3 h. The solvent was removed under reduced pressure and the residue obtained was collected by filtration and recrystallized from dioxane. Yield: 96%; mp: 286–288 °C; IR (*ν*, cm^{-1}): 3270 (NH), 3050 (CH aromatic), 1640 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) *δ* (ppm): 2.75 (s, 3H, CH_3), 7.38–7.64 (m, 9H, ArH), 7.94–7.96 (m, 2H, ArH), 8.06–8.09 (m, 2H, ArH), 8.27–8.29 (m, 2H, ArH), 10.67 (s, 1H, NH exchangeable with D_2O), 10.95 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (75 MHz, DMSO-d_6) *δ* (ppm): 11.38 (CH_3), 120.03 (2CH and C), 126.33 (CH), 127.58 (2CH), 128.52 (CH and C), 128.80 (2CH), 129.54 (2CH), 129.82 (2CH), 130.80 (2CH), 132.02 (C), 132.38 (CH), 136.91 (C), 138.49 (C), 143.38 (C), 143.69 (C), 151.01 (C), 161.78 (C=O), 165.74 (C=O); Anal. Calcd for $C_{26}H_{20}N_6O_2$ (448.48): C, 69.63; H, 4.49; N, 18.74. Found: C, 69.75; H, 4.50; N, 18.81.

4.1.12. 1,6-Diphenyl-3-methyl-5-(5-phenyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine (17)

Procedure (1): A solution of compound **16** (0.37 g, 0.8 mmol) in phosphorylchloride (5 mL) was heated under reflux at 100 °C for 5 h. After cooling, the solvent was removed in vacuo and the residue obtained was poured into an ice-water mixture and neutralized with ammonium hydroxide (20%). The solid product obtained was filtered off and recrystallized from ethanol/dioxane mixture (1:1). Yield: 80%; mp: 194–196 °C.

Procedure (2): To a cold mixture of equimolar amount of the carbohydrazide**7** (0.34 g, 1 mmol) and benzoic acid (0.12 g, 1 mmol) was added phosphorylchloride (5 mL) while stirring. The reaction mixture was then heated under reflux for 48 h, cooled, poured carefully to an equal volume of ice-water mixture followed by neutralization with solid sodium bicarbonate. The precipitate formed after standing for 1 h was filtered, washed with water and recrystallized from ethanol/dioxane mixture (1:1). The product obtained by using this method is identical in all respects (mp mixed mp and spectral data) with that obtained using method (1). Yield: 91%; mp: 194–196 °C; IR (ν, cm^{-1}): 3050 (CH aromatic); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.73 (s, 3H, CH_3), 7.35 (m, 1H, ArH), 7.49–7.64 (m, 10H, ArH), 7.79 (d, J = 8.1 Hz, 2H, ArH), 8.22 (d, J = 8.4 Hz, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 11.36 (CH_3), 120.01 (C), 122.77 (2CH), 126.54 (CH), 127.42 (2CH), 128.44 (2CH), 129.29 (4CH), 129.49 (2CH), 132.38 (C), 132.71 (2CH), 133.16 (C), 137.52 (C), 138.21 (C), 142.13 (C), 144.33 (C), 152.95 (C), 162.53 (C), 164.31 (C); Anal. Calcd for $C_{26}H_{18}N_6O$ (430.46): C, 72.55; H, 4.21; N, 19.52. Found: C, 72.46; H, 4.20; N, 19.33.

4.1.13. 1,6-Diphenyl-3-methyl-5-(1,3,4-oxadiazol-2-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine (18)

A mixture of **7** (0.34 g, 1 mmol) and triethylorthoformate (3 mL) was gently refluxed for 12 h. After cooling, the solid product formed was collected and recrystallized from ethanol. Yield: 54%; mp: 194–196 °C; IR (ν, cm^{-1}): 3100 (CH aromatic); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.74 (s, 3H, CH_3), 7.38–7.40 (m, 1H, ArH), 7.48–7.63 (m, 7H, ArH), 8.23–8.26 (m, 2H, ArH), 9.39 (s, 1H, CH oxadiazole); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 11.33 (CH_3), 120.06 (2CH and C), 128.50 (CH and C), 129.72 (2CH), 126.49 (CH), 132.61 (2CH), 133.19 (2CH), 137.19 (C), 138.21 (C), 142.32 (C), 144.28 (C), 152.85 (C), 162.27 (C), 155.06 (C); Anal. Calcd for $C_{20}H_{14}N_6O$ (354.36): C, 67.79; H, 3.98; N, 23.72. Found: C, 67.87; H, 3.89; N, 23.52.

4.1.14. 1,6-Diphenyl-*N*′-formyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carbohydrazide (19)

A mixture of carbohydrazide **7** (0.34 g, 1 mmol) and formic acid (5 mL), was heated under reflux for 48 h. After cooling, the reaction mixture was poured into ice-water and the precipitate thus obtained was filtered off and recrystallized from ethanol. Yield: 78%;mp: 236–238 °C; IR (ν, m^{-1}): 3250 (NH), 3050 (CH aromatic), 2920 (CH aliphatic), 1700 (C═O), 1660 (C═O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.71 (s, 3H, CH_3), 7.34–7.38 (m, 1H, ArH), 7.51–7.61 (m, 5H, ArH), 7.94- (m, 2H, ArH), 8.13 (s, 1H, CHO), 8.24–8.26 (d, 2H, ArH), 10.29 (s, 1H, NH), 10.93 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 11.36 (CH_3), 120.02 (C), 120.07 (2CH), 126.33 (CH), 128.49 (C), 128.62 (CH), 129.51 (2CH), 129.73 (2CH), 130.75 (2CH), 136.85 (C), 138.43 (C), 143.69 (C), 147.35 (C), 150.92 (C), 159.62 (C═O), 165.03 (C═O); Anal. Calcd for $C_{20}H_{16}N_6O_2$ (372.38): C, 64.51; H, 4.33; N, 22.57. Found: C, 64.69; H, 4.23; N, 22.60.

4.1.15. Ethyl 2-(1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-oyl)hydrazine carboxylate (20)

A solution of carbohydrazide**7** (0.34 g, 1 mmol) in ethanol (10 mL) and ethylchloroformate (2 mL) was refluxed for 1 h. After removal of the solvent, the solid product obtained was filtered off and recrystallized from ethanol. Yield: 74%; mp: 224–226 °C;IR (ν, cm^{-1}): 3300 (NH), 3000 (CH aromatic), 2900 (CH aliphatic), 1710 (C═O ester), 1670 (C═O); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.29 (t, J = 7.3 Hz, 3H, $OCH_2\underline{CH_3}$), 2.77 (s, 3H, CH_3), 4.23 (q, J = 7.2 Hz, 2H, $O\underline{CH_2}CH_3$), 6.74 (s, 1H, NH), 7.28–7.32 (m, 1H, ArH), 7.48–7.52 (m, 5H, ArH), 7.69–7.71 (m, 2H, ArH), 8.29 (d, J = 7.8 Hz, 2H, ArH), 9.09 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 11.45 ($OCH_2\underline{CH_3}$), 14.38 (CH_3), 62.41 ($O\underline{CH_2}CH_3$), 120.26 (2CH and C), 126.33 (CH), 128.03 (2CH), 129.26 (2CH), 129.44 (CH), 129.48 (2CH), 130.48 (C), 137.99 (C), 138.79 (C), 143 (C), 144 (C), 154 (C), 157 (C═O), 164.26 (CO); Anal. Calcd for $C_{22}H_{20}N_6O_3$ (416.43): Calcd: C, 63.45; H, 4.84; N, 20.18. Found: C, 63.22; H, 4.93; N, 20.09.

4.1.16. 5-Amino-1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*] pyrazine (24)

A mixture of ethyl (1,6-diphenyl-3-methyl-l*H*-pyrazolo [3,4-*b*]pyrazin-5-yl)carbamate (**23**)[13] (0.37 g, 1 mmol) and sodium hydroxide 20% (10 mL) was refluxed for 3 h. The solid product formed was collected by filtration and recrystallized from ethanol/dioxane (1:1). Yield: 90%; mp: 180–182 °C; IR (ν, cm^{-1}): 3490, 3300, 3150 (NH_2), 3050 (CH aromatic), 2900, 2820 (CH aliphatic); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H, CH_3), 4.85 (s, 2H, NH_2), 7.20–7.26 (m, 1H, ArH), 7.44–7.55 (m, 5H, ArH), 7.84 (d, J = 7.8 Hz, 2H, ArH), 8.26 (d, J = 7.8 Hz, 2H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 11.33 (CH_3), 100.53 (C), 119.62 (2CH), 125.23 (CH), 128.70 (C and CH), 129.08 (2CH), 129.70 (4CH), 137.14 (C), 139.58 (C), 141.94 (C), 149.17 (C), 154.70 (C), Anal. Calcd for $C_{18}H_{15}N_5$ (301.35): C, 71.74; H, 5.02; N, 23.24. Found: C, 71.70; H, 5.12; N, 23.19.

4.1.17. 5-Benzoylamino-1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (25)

A mixture of amine **24** (0.30 g, 1 mmol) in pyridine (10 ml) and benzoyl chloride (0.14 g, 1 mmol) was stirred at room temperature for 18 h. The reaction mixture was concentrated under reduced pressure and the solid residue obtained was filtered, washed with water, and recrystallized from ethanol. Yield: 83 %; mp: 206–208 °C; IR (ν, cm^{-1}): 3350 (NH), 3050 (CH aromatic), 2910, 2850 (CH aliphatic), 1690 (C═O).^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.72 (s, 3H, CH_3), 7.27–7.30 (m, 1H, ArH), 7.41–7.55 (m, 8H, ArH), 7.77 (d, J = 6.8 Hz, 2H, ArH), 7.85 (d, J = 5.9 Hz, 2H, ArH), 8.29 (d, J = 7.8 Hz, 2H, ArH), 8.36 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 11.43 (CH_3), 119.98 (C and 2CH), 125.84 (CH), 127.37 (2CH), 128.75 (C and CH), 128.81 (2CH), 129.18 (2CH), 129.55 (2CH), 131.30 (2CH), 132.34 (CH), 133.57 (C), 137.49 (C), 139.13 (C), 142.08 (C), 142.85 (C), 148.20 (C), 165.95 (C═O); Anal. Calcd for $C_{25}H_{19}N_5O$ (405.45): C, 74.06; H, 4.72; N, 17.27. Found: C, 74.09; H, 4.68; N, 17.39.

5. Pharmacology

Male adult Swiss–Webster mice weighing 22–30 g from the Animal House of Assiut University were used in these experiments. Mice were housed in stainless steel cages under a 12 h light/dark cycle at 25 °C and allowed water and food (laboratory show) ad libidum. The research was conducted in accordance with the internationally accepted guidelines for laboratory animals Use and Care. The experiments reported here were approved by our institutional ethics committee.

6. PTZ-induced seizures[24]

The animals were divided into groups, 8 mice each. The different compounds were injected intraperitoneally (ip) at the dose of 10 mg/kg (0.2% in corn oil), each compound to one group of mice, 20 min before ip injection of pentylenetetrazole (PTZ) at the dose of 75 mg/kg ((Minimal dose needed to induce convulsions) (1.5% solution in saline). The animals were observed for 1 h after PTZ injection for the appearance of convulsive seizures. Also, the mortality was determined 24 h later, in each group of animals. Control groups of animals were treated like wise with the pure vehicles.

Phenobarbital sodium was used as a reference drug and injected into a group of animals at the dose of 30 mg/kg, 20 min before PTZ administration.

7. Statistical analysis

The variability of results was expressed as the mean ±S.E.M. and as percentage. Statisticalanalysis of the difference between groups was done with the one-way analysis of variance (ANOVA) followed by the Dunnet's as post-hoc analysis. A *p*-value of less than 0.05 was considered statistically significant, and that of less than 0.01 was considered very significant.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.02.019.

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