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Antianexiety activity of pyridine derivatives synthesized from 2-chloro-6-hydrazino-isonicotinic acid hydrazide

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Abstract A series of oxadiazole pyridine derivatives were synthesized by using 2-chloro-6-hydrazinoisonicotinic acid hydrazide as starting material. Treatment of the hydrazide with carbon disulfide to afford the oxadiazole derivative, which was treated with 5-methyl-2-furancarbaldehyde, formic acid, acetic acid/acetic anhydride, or phthalic anhydride to yield the corresponding pyridinodiazoles and on imide. Condensation of the hydrazide with *p*-fluorobenzaldehyde in ethanol or acetic acid in the presence of sodium acetate afforded hydrazone and oxadiazole derivatives, which were acetylated and cyclized with acetic anhydride to *N*-acetyloxadiazole derivatives. The hydrazone was treated with acetic acid in the presence of sodium acetate, or bromine water/sodium acetate to give on oxadiazole, while it was cyclized with chloroacetyl chloride in the presence of *TEA* to oxoazetidinaminoisonicotinamide. Finally, condensation of the hydrazide with acid anhydrides in refluxing glacial acetic acid afforded the corresponding bisimide derivatives. The pharmacological screening showed that many of these obtained compounds have good antianexiety activity comparable to diazepam® as positive control.

Keywords 2-Chloro-6-hydrazinoisonicotinic acid hydrazide; Substituted pyridine; Oxadiazole; Oxoazetidine; Antianexiety activity.

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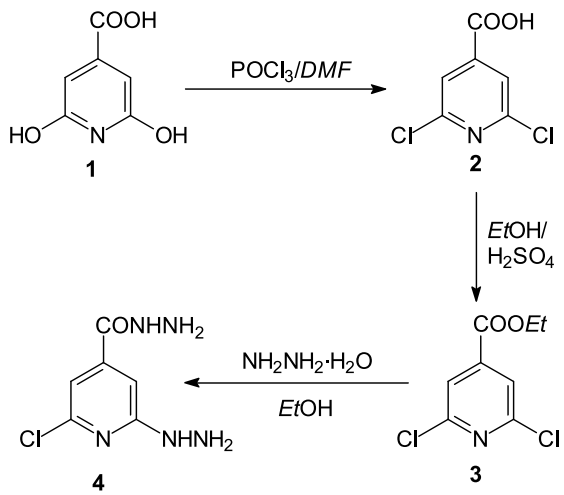
Introduction

In the previous work, acid hydrazides have been shown to be very important for the hetero-organic synthesis as key starting materials to form various classes of biologically and pharmacologically active candidates [1–7]. We have found that certain substituted isonicotinic acid hydrazide derivatives show antimicrobial activity [8, 9] and also, some of the reported pyridine derivatives can be used as antimicrobial and anti-inflammatory [10–13] and antitumor [14–16] agents. Recently, some new pyridine derivatives have been synthesized



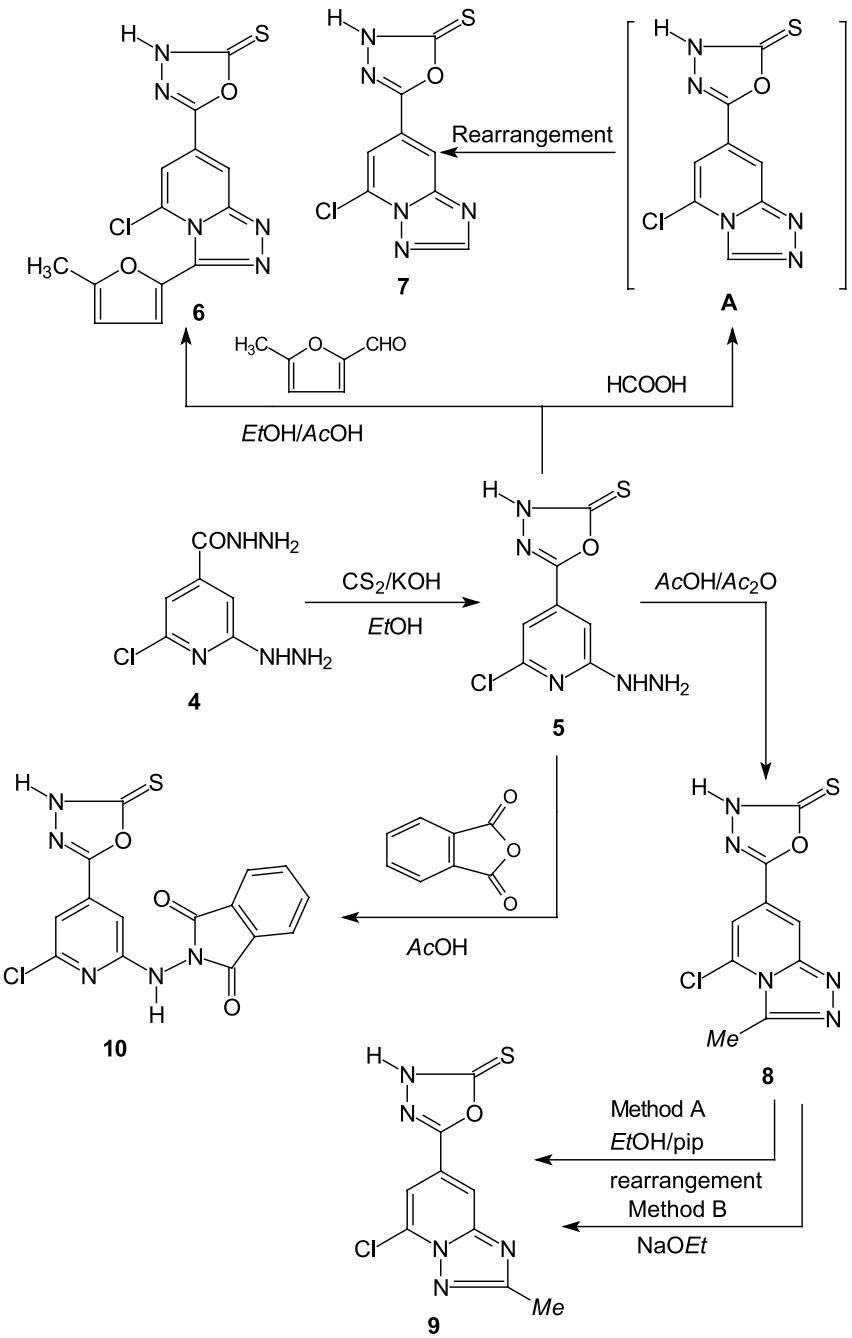
Scheme 1

and tested as analgesic, anticonvulsant, and antiparkinsonian activities [17]. In view of these observations and in continuation of our previous work in pyridine and pyrimidine chemistry, we synthesized some new heterocyclic compounds containing the pyridine moiety and tested their antianexiety activity.

Results and discussion

Chemistry

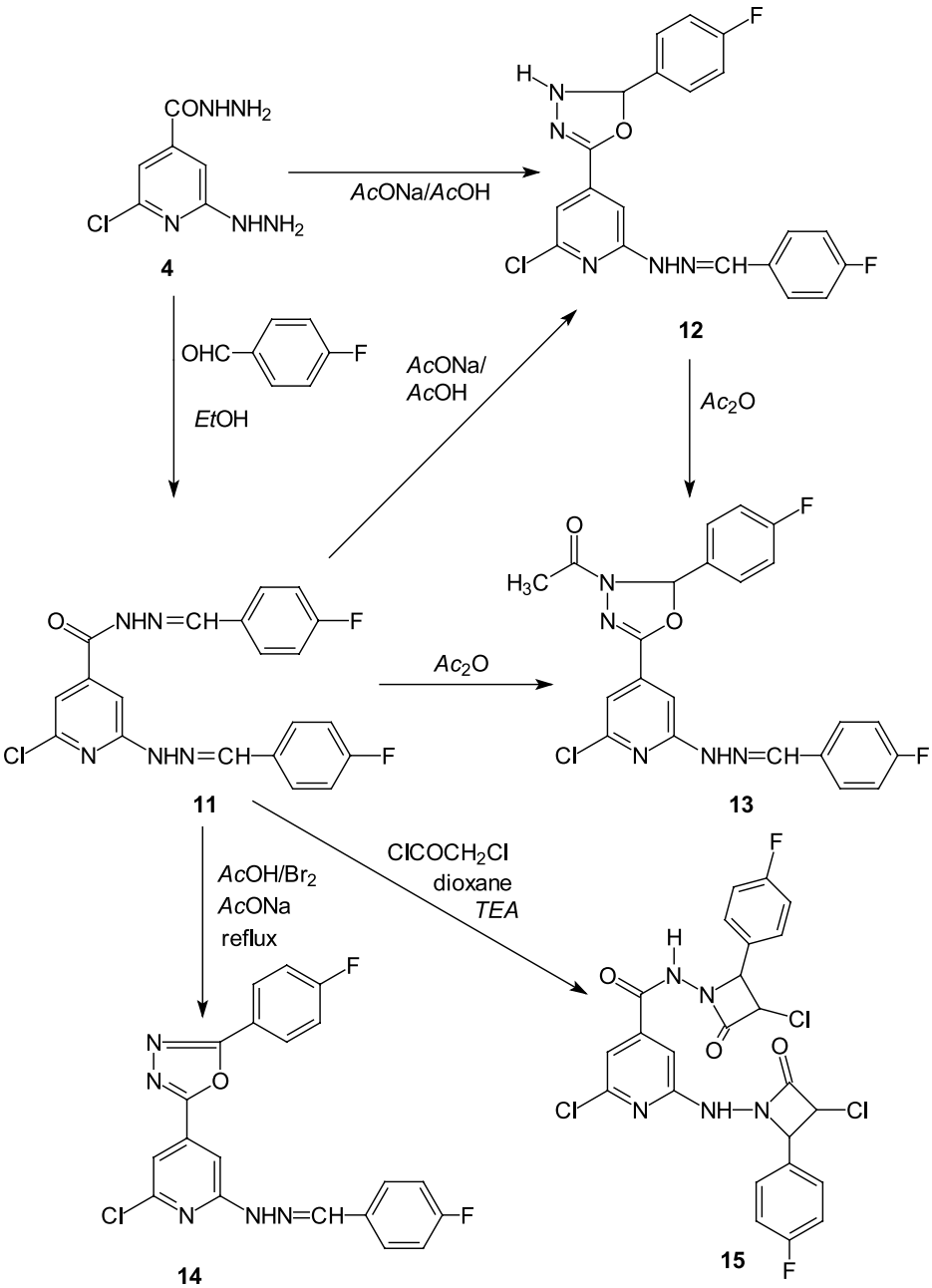
2-Chloro-6-hydrazino-isonicotinic acid hydrazide (**4**) was synthesized according to the reported procedure [8, 9]. Chlorination of 2,6-dihydroxyisonicotinic acid (**1**) with phosphorus oxychloride afforded the



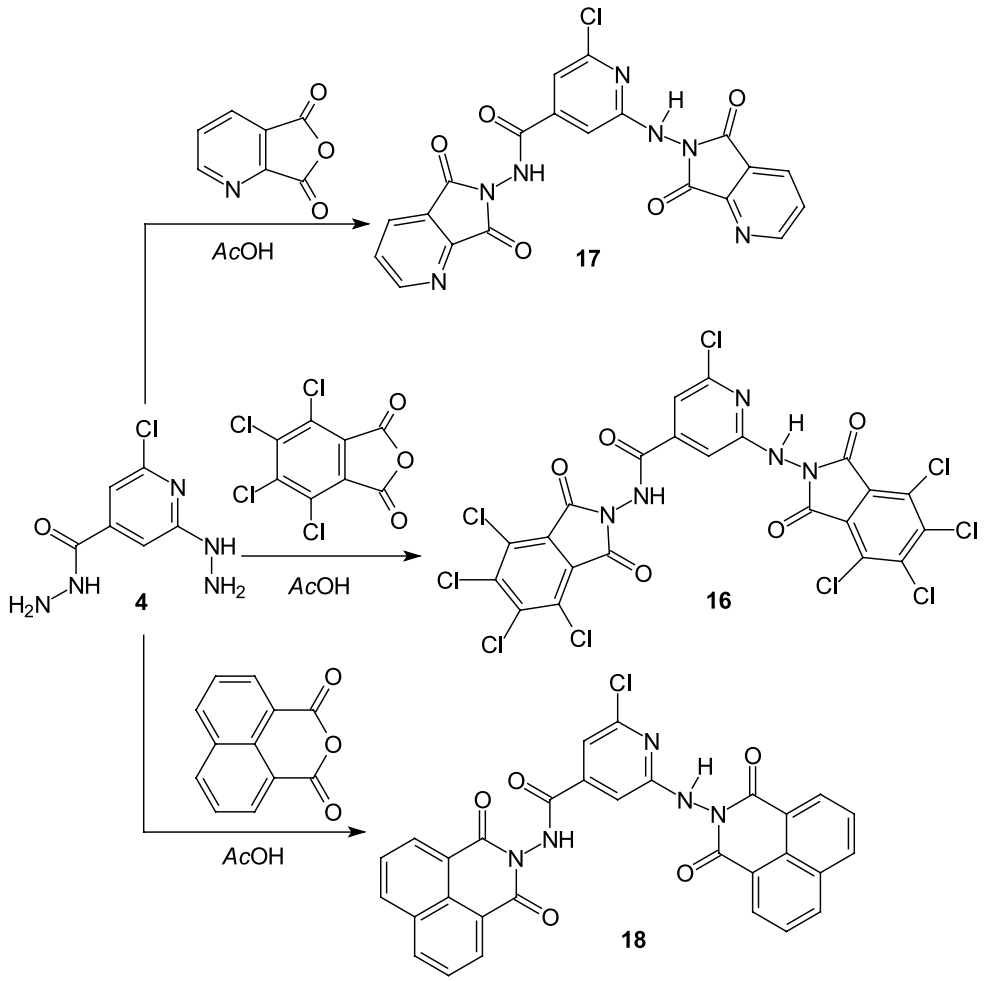
Scheme 2

corresponding 2,6-dichloroisonicotinic acid (**2**), which was esterified with absolute ethanol in the presence of concentrated sulfuric acid to afford ethyl 2,6-dichloro-isonicotinate (**3**). The ester **3** was treated with hydrazine hydrate in refluxing ethanol to afford 2-chloro-6-hydrazino isonicotinic acid hydrazide (**4**) in pure form and good yield (Scheme 1).

Treatment of **4** with carbon disulfide in the presence of ethanolic potassium hydroxide afforded the corresponding oxadiazole derivative **5**, which was reacted with 5-methyl-2-furancarbaldehyde or formic acid to afford compounds **6** and **7**, *via* the intermediate **A** [18]. Compound **5** was treated with acetic acid/acetic anhydride mixture to afford compound **8**, followed by rearrangement [19] to compound **9** by using piperidine or sodium ethoxide in ethanol as a catalyst. Also, compound **5** was reacted with phthalic anhydride in refluxing glacial acetic acid to give the imide derivative **10** (Scheme 2).



Scheme 3



Scheme 4

Condensation of compound **4** with *p*-fluorobenzaldehyde in ethanol without catalyst or acetic acid in the presence of sodium acetate afforded compounds **11** and **12**, which were cyclized and acetylated with acetic anhydride to afford compound **13**. Compound **11** was treated with acetic acid in the presence of sodium acetate and bromine water to give **14**, while **11** was cyclized with chloroacetyl chloride in the presence of *TEA* in refluxing dioxane according to the reported method [20, 21] to yield oxoazetidinaminoisonicotinamide **15** (Scheme 3).

Finally, condensation of **4** with acid anhydrides, namely, 3,4,5,6-tetrachlorophthalic anhydride, 2,3-pyridinedicarboxylic anhydride, or 1,8-naphthalene dicarboxylic anhydride in refluxing glacial acetic acid afforded the corresponding bisimide derivatives **16–18** (Scheme 4).

Table 1 Antianexiety activity of new synthesized compounds

Compound no.	Relative potency to diazepam	LD_{50}/mg kg^{-1}
4	16.20	183.54 ± 0.01
5	18.30	213.67 ± 0.02
6	17.40	172.78 ± 0.01
7	16.18	213.56 ± 0.02
8	35.16	224.45 ± 0.02
9	25.11	235.76 ± 0.02
10	12.16	226.87 ± 0.02
11	13.18	217.78 ± 0.03
12	26.11	208.65 ± 0.02
13	22.13	219.65 ± 0.01
14	21.16	256.54 ± 0.03
15	18.25	234.76 ± 0.02
16	27.16	214.76 ± 0.01
17	16.20	183.44 ± 0.01
Diazepam	1.00	108.23 ± 0.01

Pharmacological screening – antianexiety activity

Crawley et al. [22, 23] described a simple behavior model in mice to detect compounds with anxiolytic effects. Mice tend to explore a novel environment but to retreat from the aversive properties of a brightly-lit open field in a two-chambered system, where mice can freely move between a brightly-lit open field and a dark corner, animals show more crossing between the two chambers and more locomotor activity after treatment with anxiolytics.

Evaluation

Dose-response curves are obtained and the number of crossings through the partition between the light and the dark chamber are compared with total activity counts during the 10 min. All the tested compounds showed potent antianexiety activities at least 12 times more active than diazepam. Compound **8** is the most potent showing 35 times diazepam activities, the next potent compounds **16**, **12**, **9**, **13**, and **14** where there relative potency to diazepam are 27, 26, 25, 22, and 21. The relative activities of other compounds to that of diazepam lies between 12 (compound **10**) and 18 (compound **15**). The order of potency in descending order is **15**, **6**, (**17**, **4**), **7**, **11**, **10**.

Structure activity relationship (SAR)

- 3-Methyl[1,2,4]triazolo[4,3-*a*]pyridine is more potent than 2-methyl[1,2,4]triazolo[1,5-*a*]pyridine
- Chloropyridine nucleus is essential for activity
- Oxadiazole is not essential for activity; it may decrease it as in compounds **12**, **13**, and **14**.
- Triazolo moiety also not essential for activity
- Chloro derivatives increases the anti-anexity activity sharply as clear in compounds **10** and **17**.

Experimental

Chemistry

Melting points were determined on open glass capillaries using a Electrothermal IA 9000 digital melting point apparatus. Elemental analyses were performed on an Elementar, Vario EL, Microanalytical Unit, National Research Center, Cairo, Egypt and results were found within ±0.4% of the theoretical values. Infrared (IR) spectra were recorded on a Pye Unicam SP-1000 spectrophotometer using the KBr disc technique. ^{1}H NMR spectra were recorded on a Varian EM-360–270 MHz spectrometer (*DMSO*-d_6) and the chemical shifts are given in δ (ppm) downfield from *TMS* as an internal standard. Splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet. The mass spectra (MS) were measured using a VG 2AM-3F mass spectrometer. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminum sheets (Type 60 F_{254}, Merck) and the spots were detected by exposure to a UV lamp at $\lambda = 254$ nm for few seconds. 2-Chloro-6-hydrazinoisonicotinic acid hydrazide (**4**) was synthesized according to Refs. [8, 9].

5-(2-Chloro-6-methylpyridin-4-yl)[1,3,4]oxadiazole-2-thiol (**5**, $C_7H_6ClN_5OS$)

To a solution of 2.01 g **4** (10 mmol) in 25 cm^3 ethanolic potassium hydroxide (5%), 1.5 cm^3 carbon disulphide (20 mmol) was added. The reaction mixture was stirred for 1 h at room temperature and another 6 h under reflux, after cooling, it was poured onto ice-water and acidified with HCl ($pH \sim 3$). The formed solid was filtered off, washed with water, dried, and crystallized to give **5** (95%). Mp 286°C (*DMF*); IR (film): $\bar{\nu} = 3410$–3330 (NH, NH_2), 1600 (C=N), 1260 (C=S) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 6.80$ (s, NH_2 exchangeable with D_2O), 8.10, 8.18 (2s, pyr-H), 9.60, 14.90 (2s, 2NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 243$ [M$^+$, 20] and at 64 [100, base peak].

5-[5-Chloro-3-(5-methylfuran-2-yl)[1,2,4]thiazolo[4,3-a]pyridin-7-yl][1,3,4]oxadiazole-2-thiol (**6**, $C_{13}H_8ClN_5O_2S$)

To a solution of 2.4 g **5** (10 mmol) in 30 cm^3 absolute ethanol containing 3 drops of glacial acetic acid, 1.1 g of 5-methyl-2-furancarbaldehyde (10 mmol) was added. The reaction mixture was heated under reflux, after cooling, the solid was filtered off, dried and crystallized to afford **6** (60%). Mp >300°C (*DMF*); IR (film): $\bar{\nu} = 3380$ (NH), 1598 (C=N), 1260 (C=S) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 2.10$ (s, CH_3), 5.80, 6.40 (2d, 2 furon-H), 8.32, 8.38 (2s, pyr-H), 9.60 (s, NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 333$ [M$^+$, 10] and at 76 [100, base peak].

5-(5-Chloro[1,2,4]triazolo[1,5-a]pyridin-7-yl)[1,3,4]-oxadiazole-2-thiol (**7**, $C_8H_4ClN_5OS$)

A solution of 2.4 g **5** (10 mmol) in 20 cm^3 formic acid was heated under reflux for 8 h. The reaction mixture was cooled then poured onto ice-water, the obtained solid was filtered off, washed with water, dried, and crystallized to afford **7** (67%). Mp >300°C (*DMF*); IR (film): $\bar{\nu} = 3320$ (NH), 1605 (C=N), 1262 (C=S) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 7.78$–8.32 (m, 2 pyr-H, triazole-H), 9.50 (s, NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 253$ [M$^+$, 15] and at 57 [100, base peak].

5-(5-Chloro-3-methyl[1,2,4]triazolo[4,3-a]pyridin-7-yl)-[1,3,4]oxadiazole-2-thiol (**8**, $C_9H_6ClN_5OS$)

A solution of 2.4 g **5** (10 mmol) in 20 cm^3 *Ac*OH/*Ac*$_2$O as a mixture (1/1) was refluxed for 10 h. The reaction mixture was cooled and the obtained red solid was filtered off,

washed with water, dried, and crystallized to give **8** (62%). Mp 252°C (*DMF*); IR (film): $\bar{\nu} = 3340$ (NH), 1609 (C=N), 1256 (C=S) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 1.95$ (s, CH_3), 7.78, 8.12 (2s, 2 pyr-H), 9.60 (s, NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 266$ [M^+, 2] and at 251 [100, base peak].

5-(5-Chloro-2-methyl[1,2,4]triazolo[1,5-a]pyridin-7-yl)-[1,3,4]oxadiazole-2-thiol (**9**, $C_9H_6ClN_5OS$)
Method A. A solution of 2.6 g **8** (10 mmol) in 20 cm^3 absolute ethanol in the presence of few drops of pipridine was refluxed for 6 h. The reaction mixture was evaporated under reduced pressure, the residue was triturated with ether, the formed solid was filtered off, dried and crystallized to give **9** (68%). Mp 268°C (*Et*OH/ether); IR (film): $\bar{\nu} = 3409$ (NH), 1610 (C=N), 1257 (C=S) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 1.90$ (s, CH_3), 7.90, 8.30 (2s, 2 pyr-H), 9.75 (s, NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 266$ [M^+, 2] and at 171 [100, base peak].

Method B. A mixture of 2.6 g **8** (10 mmol) and sodium ethoxide (0.5 g sodium metal in 30 cm^3 absolute ethanol) was heated under reflux for 4 h. After cooling, the reaction mixture was concentrated under reduced pressure, poured onto water, acidified with 1 *N* HCl. The product was extracted with ethyl acetate, dried over sodium sulphate anhydrous, evaporated under reduced pressure, the residue was solidified with ether, filtered off, dried, and crystallized to give **9** (72%).

2-[6-Chloro-4-(5-mercapto[1,3,4]oxadiazol-2-yl)pyridin-2-ylamino)isoindole-1,3-dione (**10**, $C_{15}H_8ClN_5O_3S$)
A mixture of 2.4 g **5** (10 mmol) and 1.48 g of phthalic anhydride (10 mmol) in 50 cm^3 glacial acetic acid was heated under reflux for 6 h. The reaction mixture was concentrated under reduced pressure, the obtained solid was filtered off and crystallized to yield **10** (72%). Mp >300°C (dioxane); IR (film): $\bar{\nu} = 3331$ (NH), 1600 (C=N), 1258 (C=S) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 7.95$–8.42 (m, 2 pyr-H, 4H–Ar–H), 8.65, 9.62 (2s, 2NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 373$ [M^+, 10] and at 313 [100, base peak].

2-Chloro-6-[N′-(4-fluorobenzylidene)hydrazino]isonicotinic acid (4-fluorobenylidine)hydrazide (**11**, $C_{20}H_{14}ClF_2N_5O$)
A mixture of 2.10 g **4** (10 mmol) and 2.4 g of *p*-flourobenzaldehyde (20 mmol) in 50 cm^3 absolute ethanol was refluxed for 3 h. After, cooling, the separated solid was filtered off, dried, and crystallized to give **11** (98%). Mp 279°C (dioxane); IR (film): $\bar{\nu} = 3260$–3230 (NH), 1665 (C=O), 1602 (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 7.65$–8.05 (m, Ar–H), 8.25–8.42 (m, 2 pyr-H, 2H hydrazone-H), 11.60, 12.20 (2s, 2 NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 413$ [M^+, 40] and at 171 [100, base peak].

2-Chloro-N-(1,3-dioxo-1H,3H-benzo[de]isoquinolin-2-yl)-6-(1,3-dioxo-1H,3H-benzo[de]iso-quinolin-2-ylamino)iso-nicotinamide (**12**, $C_{20}H_{14}ClF_2N_5O$)
Method A. A mixture of 2.1 g **4** (10 mmol), 2.4 g *p*-fluorobenzaldehyde (20 mmol) and 1.2 g anhydrous sodium acetate (15 mmol) in 30 cm^3 glacial acetic acid was refluxed for 10 h. The reaction mixture was concentrated under reduced pressure, poured onto water, the formed solid was filtered off, washed with water, dried and crystallized to yield **12** (80%). Mp 231°C (*Et*OH); IR (film): $\bar{\nu} = 3409$ (NH), 1605 (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 5.65$ (s, CH-oxadiazole-H), 7.20–8.10 (m, Ar–H), 8.24–8.32 (m, 2 pyr-H, hydrazone-H), 11.55, 12.15 (2s, 2NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 413$ [M^+, 2] and at 171 [100, base peak].

Method B. A mixture of 4 g **11** (10 mmol) and 1.2 g anhydrous sodium acetate (15 mmol) in 20 cm^3 glacial acetic acid was refluxed for 3 h. The reaction mixture was concentrated under reduced pressure, poured into water. The obtained solid was filtered off, dried, and crystallized to give **12** (85%).

1-[5-{2-Chloro-6-[2-(4-fluorophenyl)vinylamino]pyridin-4-yl}-2-(4-fluorophenyl)-[1,3,4]oxa-diazol-3-yl]ethanone (**13**, $C_{22}H_{16}ClF_2N_5O_2$)
Method A. A solution of 2.10 g **11** (5 mmol) in 30 cm^3 acetic anhydride was heated under reflux for 6 h. The reaction mixture was concentrated under reduced pressure and cooled, the separated solid was filtered off, washed with petroleum ether, dried, and crystallized to yield **13** (76%). Mp 195°C (dioxane); IR (film): $\bar{\nu} = 3320$ (NH), 1704 (C=O), 1605 (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 2.10$ (s, CH_3), 5.62 (s, CH-oxadiazole-H), 7.75–8.00 (m, Ar–H), 8.18–8.34 (m, 2 pyr-H, hydrazone-H), 10.95 (s, NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 455$ [M^+, 65] and at 122 [100, base peak].

Method B. A solution of 4 g **12** (10 mmol) in 15 cm^3 acetic anhydride was refluxed for 2 h. The separated solid was filtered off, dried, and crystallized to give **13** (70%).

N-{6-Chloro-4-[5-(4-fluorophenyl)[1,3,4]oxadiazol-2-yl]-pyridin-2-yl}-N′-(4-fluorobenzylidine)-hydrazine (**14**, $C_{20}H_{12}ClF_2N_5O$)
To a suspension solution of 4.13 g **11** (10 mmol), 1.2 g anhydrous sodium acetate (15 mmol) in 20 cm^3 glacial acetic acid, 1.6 g bromine (10 mmol) in 10 cm^3 glacial acetic acid was added. The reaction mixture was refluxed for 1 h, after cooling, it was poured onto water, the formed solid was filtered off, washed with water, dried, and crystallized to afford **14** (85%). Mp 266°C (*Et*OH); IR (film): $\bar{\nu} = 3328$ (NH), 1605 (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 7.82$–8.5 (m, Ar–H), 8.22–8.30 (m, 2 pyr-H, hydrazone-H), 11.24 (s, NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 411$ [M^+, 100, base peak].

2-Chloro-N-[3-chloro-2-(4-fluorophenyl)-4-oxoazetidin-1-yl]-6-[3-chloro-2-(4-fluorophenyl)-4-oxoazetidin-1-ylamino]-isonicotinamide (**15**, $C_{24}H_{16}C_{l3}F_2N_5O_3$)
To a solution of 4.1 g **11** (10 mmol) in 20 cm^3 dioxane containing few drops of *TEA* as a catalyst, 2.24 cm^3 chloroacetyl chloride (20 mmol) was added drop-wise with stirring at room temperature. The solution was heated

under reflux for 3 h, after cooling, the formed solid was filtered off and crystallized to yield **15** (96%). Mp 260°C (*DMF*); IR (film): $\bar{\nu} = 3280–3230$ (NH), 1665, 1660 (C=O), 1605 (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta =$ 6.65, 6.75 (2s, 4CH), 7.72–7.96 (m, Ar–H), 8.24, 8.32 (2s, pyr-H), 9.40, 10.60 (2s, 2NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 579$ [M^+, 10] and at 479 [100, base peak].

*Synthesis of bisimide derivatives **16–18***

A mixture of 2.10 g **4** (10 mmol) and acid anhydride, namely, 1,2,4,5-tetrachlorophthalic anhydride, 2,3-pyridinedicarboxylic anhydride, or 1,8-naphthalenedicarboxylic anhydride (20 mmol) in 50 cm^3 glacial acetic acid was heated under reflux for 6 h. The reaction mixture was concentrated under reduced pressure, the residue was solidified with ether and filtered off to yield **16** (65%), **17** (72%), and **18** (68%).

2-Chloro-N-[4,5,6,7-tetrachloro-1,3-dioxo-1,3-dihydro-isoindol-2-yl)-6-(4,5,6,7-tetrachloro-1,3-dioxo-1,3-dihydro-isoindol-2-ylamino)isonicotinamide (**16**, $C_{22}H_4Cl_9N_5O_5$)

Mp 256–258°C (*Ac*OH/H_2O); IR (film): $\bar{\nu} = 3331$ (NH), 1665 (C=O), 1600 (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 8.15$, 8.24 (2s, pyr-H), 9.62, 11.50 (2s, 2NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 737$ [M^+, 12] and at 438 [100, base peak].

2-Chloro-N-[5,7-dioxo-5,7-dihydropyrrolo[3,4-b]pyridin-6-yl)-6-(5,7-dioxo-5,7-dihydropyrrolo-[3,4-b]pyridin-6-ylamino)isonicotinamide (**17**, $C_{20}H_{10}ClN_7O_5$)

Mp 234–236°C (*Ac*OH/H_2O); IR (film): $\bar{\nu} = 3338$, 3295 (NH), 1668 (C=O), 1605 (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 8.15–8.85$ (m, pyr-H), 9.65, 13.35 (2s, 2NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 463$ [M^+, 100, base peak].

2-Chloro-N-[1,3-dioxo-1H,3H-benzo[de]isoquinolin-2-yl)-6-(1,3-dioxo-1H,3H-benzo[de]iso-quinolin-2-ylamino)-isonicotinamide (**18**, $C_{30}H_{16}ClN_5O_5$)

Mp > 300°C (*Ac*OH/H_2O); IR (film): $\bar{\nu} = 3342$, 3320 (NH), 1665 (C=O), 1610 (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 7.95$, 8.10 (2s, pyr-H), 8.24–8.75 (m, Ar–H), 9.55, 13.20 (2s, 2NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 561$ [M^+, 8] and at 350 [100, base peak].

Pharmacological screening

Antianexiety activity – procedure

The testing apparatus consists of a light and a dark chamber divided by a photocell-equipped zone. A polypropylene animal cage $44 \times 21 \times 21$ cm is darkened with black spray overone-third of its surface. A partition containing a 13 cm long $\times 5$ cm high opening separates the dark one third from the bright two third of the cage. The cage rests on an Animex® activity monitor, which counts total 10 locomotor activity. As electronic system using four sets of photocells across the partition automatically counts movements through the partition and clocks the time spent in the light and dark compartments. Naïve male albino mice with a body weight between 18 and 25 g are placed into the cage, the animals are treated 30 min before the experiment with the test drugs or the vehicle intraperitoneally, and are then observed for 10 min [24, 25].

Determination of LD_{50}

Determination of median lethal dose (*Lorke* 5) Phase 1: Three-groups of three mice/group. One dose was given to each group intraperitoneally. The treated mice were monitored for 24 h for mortality and general behavior.

Phase 2: After 24 h 3–4 groups of one mouse were given doses based on the findings of phase 1, intraperitoneally. The mice were again monitored for 24 h. The geographic mean of the least dose that killed mice and the highest dose that did not kill mice was taken as the median lethal dose [26–28] (Table 2).

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