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Original article

Synthesis of new pyrazole derivatives and their anticancer evaluation

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ABSTRACT

A series of functionally substituted pyrazole compounds have been synthesized and evaluated *in vitro* for their antiproliferative effects on a panel of 60 cellular lines, according to the National Cancer Institute screening protocol. Three of the 12 tested compounds showed moderate antitumor activity, one of them being chosen for the 5-dose assay and presented $\log GI_{50}$ values up to −5.75.

1. Introduction

Pyrazole showed promising anticancer effects and in the 1960s, it was evaluated in Phase I studies as an antitumor agent in man, but, even in doses of 0.15 mmol/kg/day it proved too toxic for human use because of development of signs of hepato-toxicity [1]. Trying to overcome this toxicity, 1-carboxamidopyrazole and 1-thiocarbamoylpyrazole were synthesized and they showed significant anticancer effects on animal experiments, but failed to pass the clinical evaluation [2,3]. In search for better antitumor treatment, a large series of pyrazole derivatives were synthesized and tested over the years, the use of this powerful pharmacophore being very popular and modern [4–6].

In the last decade several pyrazole derivatives proved to have potent anticancer action by the inhibition of the cyclin-dependent kinases. (CDKs). CDKs are members of the large family of protein kinases and are responsabile for the eukariotic cell cycle regulation; they are intesively studied for their cancer implication [7–11]. Based on the model of 1-carboxamidopyrazole, a series of pyrazole derivatives containing an urea scaffold proved to have antiproliferative effects by inhibiting Aurora kinase activity [12,13]. Some other protein kinases are targets of pyrazole based derivatives, some aryl- and heteroaryl-substituted pyrazole act as inhibitors of the transforming growth factor-beta type I receptor kinase domain [14]. The anticancer effects of some pyrazole amide derivatives are mediated by inhibition of the Ras–Net pathway and microtubule depolymerization [15].

Based on the model of these aforementioned pyrazole urea derivatives, 1-thyocarbamoylpyrazole and some antiproliferative acyl thioureas [16–18], we designed and synthesized some chimeric thiourea-pyrazole derivatives. Fig. 1 shows relevant antitumor pyrazole derivatives, a pattern for our compounds design.

2. Results and discussion

2.1. Chemistry

The new compounds were synthesized by the general methods outlined in Schemes 1 and 2. The precursor 1-methyl-1H-pyrazole-4-carboxylic acid (1) was converted into the 1-methyl-1H-pyrazole-4-carbonyl chloride (2) using thionyl chloride as chlorination reagent and was treated with ammonium isothiocyanate to afford 1-methyl-1H-pyrazole-4-carbonyl isothiocyanate (3). This was converted into the corresponding N-(1-methyl-1H-pyrazole-4-carbonyl)-N′-(aryl)-thioureas (4a–j) by adding various substituted anilines and refluxing in dry acetone. The 5-(1-methyl-1H-pyrazol-4-yl)-1-phenyl-1,2-dihydro-1,2,4-triazole-3-thione (5) was obtained in one step reaction from 1-methyl-1H-pyrazole-4-carbonyl isothiocyanate and phenylhydrazine. The solvent was changed to acetonitrile to prevent the hydrozone formation.

The structures of the synthesized compounds were confirmed by spectral data. For the compounds 4a–j the ^{1}H NMR spectra

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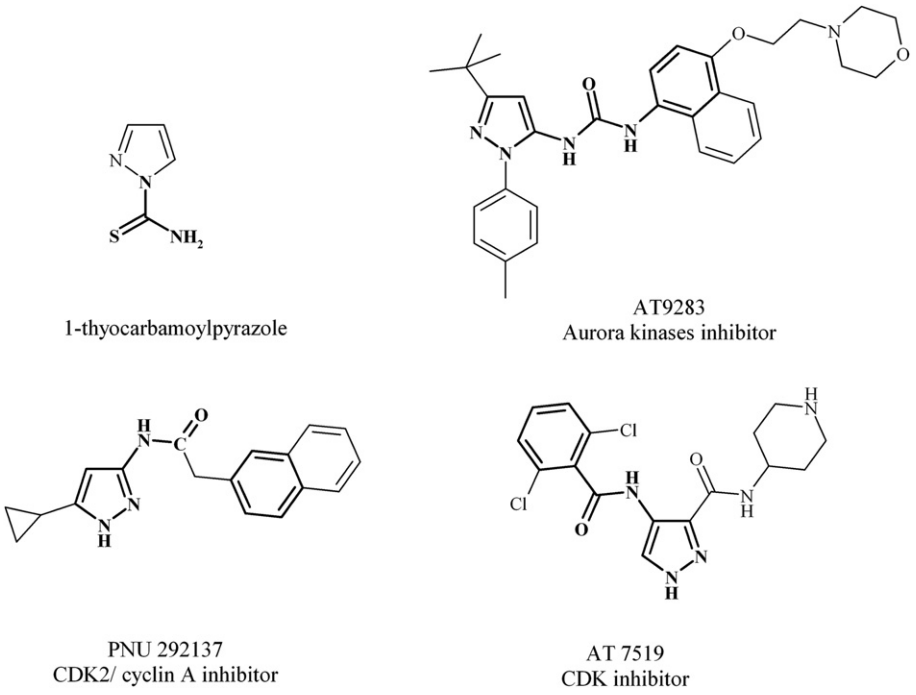


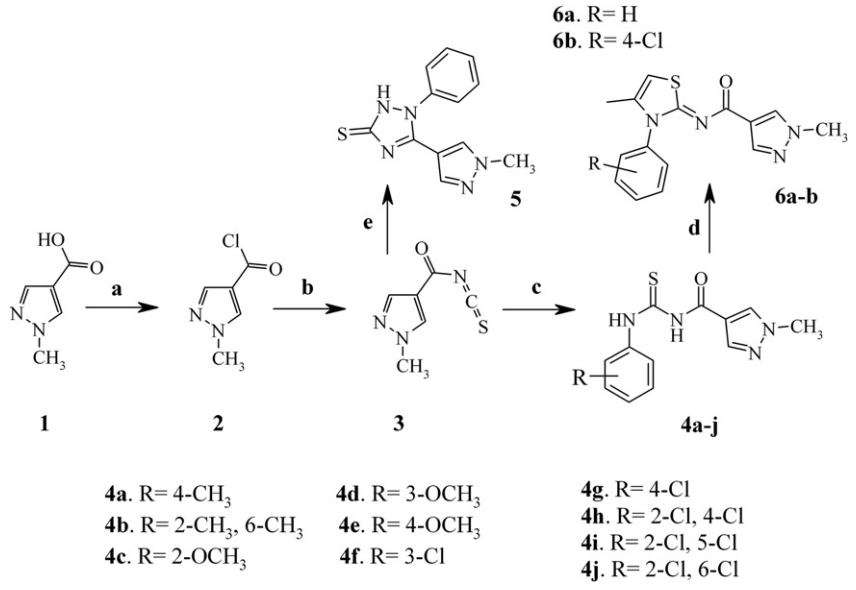
Fig. 1. Structures of antitumor pyrazoles as rational compounds design template.

showed two broad singlets for the thiourea moiety, 12.50–11.99 ppm (–NH–C=S) and 11.60–11.20 ppm (–NH–C=O). The hydrogens of the pyrazole ring appeared as two singlets ~8.59 ppm and ~8.21 ppm, values indifferent to the aryl substitution. These values are lower in the compound 5, 8.32 ppm and 8.00 ppm. In the ^{13}C NMR spectra the acyl thiourea carbons appeared at 181.1–178.3 ppm (C=S) and 163.8–162.9 ppm (C=O). The pyrazole ring appeared as 141.1–140.3 ppm (C-5), 136.3–134.5 ppm (C-3) and 116.1–115.4 ppm (C-4). The N–CH_3 presented a signal in the range of 39.7–39.0 ppm.

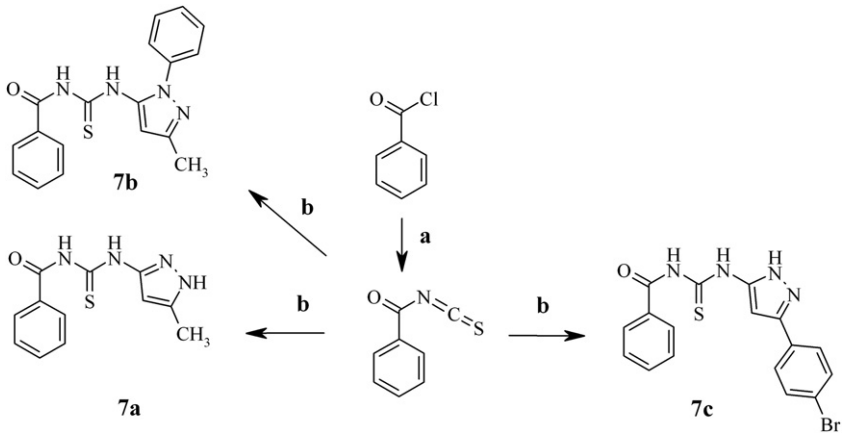
In order to better evaluate the structure–activity relationship we synthesized some thiazol-2(3H)-ylidene derivatives (6a–b) by treating the new acyl thioureas with bromoacetone under basic conditions [19]. The pyrazole hydrogens appeared with lower values in the ^1H NMR spectra, ~7.78 ppm and ~7.48 ppm, because of the internal H-bond disappearance. Distinctive is the thiazolylidene hydrogen singlet appearing at 6.81–6.75 ppm.

By the structural rearrangement of the compounds 4 skeleton we designed the compound 7a and the structural analogues 7b–c. The compounds were synthesized transforming benzoyl chloride in benzoyl isothiocyanate which was treated with the corresponding pyrazole amine.

The compounds 7a–c ^1H NMR spectra present two broad singlets for the thiourea group, 13.17–12.46 ppm and 11.80–11.56 ppm and



Scheme 1. Reagents: (a) $SOCl_2$, $C_2H_4Cl_2$, reflux; (b) NH_4SCN, $(CH_3)_2CO$, reflux; (c) R–C_6H_4–NH_2, $(CH_3)_2CO$, reflux; (d) $(C_2H_5)_3N$, $BrCH_2COCH_3$; (e) $C_6H_5NHNH_2$, CH_3CN, reflux.



Scheme 2. Reagents: (a) NH_4SCN, $(CH_3)_2CO$, reflux; (b) $(CH_3)_2CO$, reflux.

one broad singlet 13.37–13.10 ppm for the pyrazole NH, in compounds 7a and 7c, the pyrazole CH as a singlet and the aromatic hydrogens in the range of 7.99–7.38 ppm. In the ^{13}C NMR spectra the acyl thiourea carbons appeared at 180.1–170.0 ppm (C=S) and 168.8–168.0 ppm (C=O). The pyrazole moiety appeared as 147.8–140.8 ppm (C-5), 147.8–138.3 ppm (C-3) and 102.9–96.1 ppm (C-4).

The new acyl thioureas are characterized by IR absorptions in the ranges of 3350–3300, 3250–3200 for the free and associated NH, 1650–1675 for carbonyl and 1230–1250 cm^{-1} for thiocarbonyl group. In the thiazoliden-2-imines (6a–b) the carbonyl vibration band shifts to lower values because of the conjugation with the imine bond.

The compound's purity was certified by elemental analyses, the results being within ±0.4 of the theoretical values.

2.2. Anticancer activity

A number of 12 compounds were selected by the National Cancer Institute (NCI) for *in vitro* anticancer evaluation on 60 different human tumor cell lines, representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney. The screening was performed according to the NCI protocol. The results for each tested compounds are reported as growth percentages compared with the untreated control cells after 48 h of drug exposure. The compounds were tested initially at a single high dose of 10^{-5} M and one compound was chosen and tested on 5-dose assay using 1 log serial dilutions from 10^{-8} to 10^{-4} M. The results are reported as percent growth of the tested cells comparing with the control cells values. The screening results are presented in Table 1. The positive values under 100 show a growth inhibitory effect and the negative values indicate a cytotoxic effect.

Statistically examining the growth inhibition percent results (GI) for the 12 tested compounds, using the hierarchical cluster analysis based on the Euclidean distances between variables ($D_{x,y}$) and Ward's method we obtained the dendogram presented in Fig. 2. The Euclidean distances are also used as dissimilarity measure to compare each compound's results with the control. This method permits a better classification based on the anticancer effect and the identification of some structure–activity relationship.

Based on this cluster classification, we can divide the compounds in two major groups, one (A) containing the N-(1-methyl-1H-pyrazole-4-carbonyl)-N′-(aryl)-thioureas and the structurally similar compound 5, with low Euclidean distances to the control values, and the other (B) containing the thiazolylidene derivatives and the pyrazole amine thioureas, presenting high Euclidean distances to the control. This effect-based classification is similar to the chemical classification, emphasizing on the structure's importance for the antitumor action.

The tested N-(1-methyl-1H-pyrazole-4-carbonyl)-N′-(aryl)-thioureas show little effect on the cancer cells, excepting some few cell lines, which show a growth inhibition over 50%. By including the thiourea moiety in a 1,2,4-triazole-3-thione ring, as in compound 5, the inhibitory effect dissappears.

However, the transformation of the thiourea group in a thiazol-2(3H)-ylidene ring significantly improves the anticancer effects. The compounds 6a and 6b share similar anticancer profiles, the best action being noticed against ACHN renal cells, DU-145 prostate cancer, on SK-MEL-2 melanocytes and the colon cells HCT-15 and HT29.

Using the retro-inversion of thiourea substituents of compound 4a, the resulting 7a has insignifiant effects on the cellular growth of most cell lines tested, except for four lines that are strongly stimulated, with growth precentages in the range of 161.3 and 209.2. The anticancer effect is greatly increased by adding a phenyl group on the position 1 of the pyrazole ring (7b), the stimulatory effect being significaly reduced, and the best inhibitory effects are noticed on the leukemia cells. This inversion of acyl and alkyl function of the pyrazole ring on thiourea moiety is important for the antitumor action.

Table 1
Antitumor activity of compounds at 10^{-5} M against cancer cells.

Test compound	Range of growth %		Mean growth %	Most sensitive cells
	min	max		
7c	−19.9	116.3	70.1	−19.9(TK−10/R) 1.0 (HCT-116/C) 8.9 (NCI-H522/L)
6b	−11.8	119.8	80.5	−11.8(ACHN/R) 0.3 (DU-145/P)
6a	−15.7	128.1	82.5	−15.7(ACHN/R) 1.6 (DU-145/P)
7b	22.6	125.7	84.6	22.6 (MDA-MB-435/M)
4g	47.2	128.9	91.1	47.2 (NCI-H522/L)
4a	62.5	120.1	101.0	62.5 (NCI-H522/L)
4h	79.7	190.5	101.0	79.7 (UACC-62/M)
4e	68.7	118.9	102.2	68.7 (HOP-92/L)
4b	40.5	138.7	105.9	40.5 (MOLT-4/Lk)
4j	69.7	148.9	107.4	69.7 (CAKI-1/R)
7a	89	209.2	109.9	–
5	95	143.5	110.5	–

R – renal cancer, C – colon cancer, L – non-small cell lung cancer, P – prostate cancer, M – melanoma, Lk – leukemia, B – breast cancer, N – CNS cancer, O – ovarian cancer.

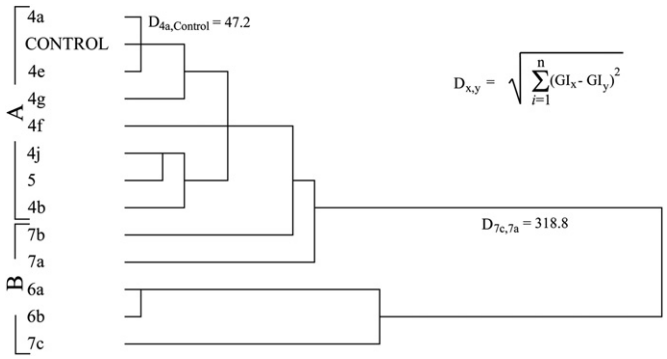


Fig. 2. Hierarchical cluster classification of the compounds.

The best antitumor action is achieved for the compound 7c, a close analog of 7b, having an additional bromine atom and a pyrazole –NH– group free to form hydrogen bonds. The compound has promising antitumor effects, with growth inhibition percents over 50% on 12 cell lines and was chosen for advance tests on a 5 fold range of concentrations. The results are presented in Table 2 and are expressed as $logGI_{50}$, logTGI and $logLC_{50}$. These are logarithmized molar concentration producing a 50% growth inhibition (GI_{50}), a total growth inhibition (TGI) and a 50% cellular death (LC_{50}), respectively. The values were calculated if the target level of activity was reached, if not the value is expressed as more than the highest tested concentration.

The compound 7c presented $logGI_{50}$ values under −4 in 43 of the 60 cancer lines, values ranging from −4.12 to −5.75, with an average of −5.02. The best results were recorded on the ovarian cancer cells with values ranging from −4.66 to −5.37, with an average of −5.14, and the renal cancer cells, with values between −4.40 and −5.49. A number of 24 tested cancer cell lines presented logTGI values under −4, the best value being noted on the colon cancer cells HCT-116. Only in 11 cell lines, compound 7c registred $logLC_{50}$ values under −4.

Considering the design premises of these compounds, we expect the antitumor effect to be created by CDKs inhibition. Comparing the stimulatory effect of compound 7a to the inhibitory effect of 7c we think the changing of the methyl group in 7a with a 4-bromophenyl in 7c offered the lipophilic and bulky area neccesary for the CDKs inhibition. Following the model of 6a–b, the future transformation of the compounds 7b–c in thiazolylidene derivatives may be very useful.

Table 2
The most sensitive cancer cells to 7c.

Cancer type	Most sensitive cells	logGI50	logTGI	logLC50
Leukemia	SR	−4.93	−4.39	>−4
Non-Small cell lung cancer	A549/ATCC	−5.63	−4.85	−4.1
	NCI-H322M	−5.35	−4.35	>−4
	NCI-H23	−5.03	−4.30	>−4
Colon cancer	HCT-116	−5.75	−5.32	−4.61
CNS cancer	SF-539	−5.47	>−4	>−4
	SNB-75	−5.21	−4.57	−4.05
	SF-295	−5.01	−4.44	>−4
Melanoma	LOX IMVI	−5.26	>−4	>−4
	MALME-3M	−5.18	−4.04	>−4
Ovarian cancer	OVCAR-4	−5.37	−4.62	>−4
	OVCAR-8	−5.33	−4.68	−4.16
	NCI/ADR-RES	−5.31	−4.20	>−4
	OVCAR-3	−5.30	−4.69	−4.15
	SK-OV-3	−5.14	−4.66	−4.28
Renal cancer	RXF 393	−5.50	−4.82	−4.32
	786–0	−5.49	−4.93	−4.32
	UO-31	−5.39	−4.67	−4.07
Prostate cancer	DU-145	−5.03	>−4	>−4
Breast cancer	HS 578T	−5.56	−4.47	>−4
	MDA-MB-231/ATCC	−5.42	−4.65	>−4

3. Conclusion

A set of pyrazole based small molecules were designed and synthesized as potential antitumor agents. They were tested in an *in vitro* assay against 60 types of human cancer cells to evaluate their anticancer effects. Three compounds presented significant growth inhibitory effects on the tested cancer cells. The preliminary SAR reveals that the substituted pyrazolyl thiourea derivatives are more active then the related N-[(1-methyl-1H-pyrazole-4-yl) carbonyl]-thiourea derivatives. However, the transformation of the latter in thiazolylidene derivatives enhance the anticancer effects. The promising results obtained with the compound N-benzoyl-N′-(3-(4-bromophenyl)-1H-pyrazol-5-yl)-thiourea (7c) and the SAR results encourage us to develop new antitumor substances of the pyrazole derivatives class.

4. Experimental

4.1. Chemistry

All starting materials and solvents were purchased from common commercial suppliers and used without purification, unless otherwise noted. The acetone was dried over potassium carbonate and distillated, and the ammonium thiocyanate by heating at 100 °C. The melting points were measured in open capillary tubes on an Electrothermal 9100 apparatus and are uncorrected. The elemental analyses were performed on a Perkin Elmer CHNS/O Analyser Series II 2400 apparatus. The NMR spectra were recorded on a Varian Gemini 300BB instrument at room temperature, operating at 300 MHz for ^{1}H and 75.075 MHz for ^{13}C. The chemical shifts were recorded as δ values in ppm units downfield to tetramethylsilane, used as internal standard. The coupling constants values are reported in hertz and the splitting patterns are abbreviated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; b, broad. The IR spectra were recorded on a JASCO FT/IR-4200 spectrometer with a diamond crystal ATR PRO450-S accessory.

4.1.1. General synthesis procedure for compounds 4a–j

A solution of 1-methyl-1H-pyrazole-4-carboxylic acid (0.1 mol) in anhydrous 1,2-dichlorethane is refluxed for 3 h with thionyl chloride (14.5 mL, 0.2 mol). The solvent and the excess thionyl chloride are removed by reduced pressure distillation. The raw obtained 1-methyl-1H-pyrazole-4-carbonyl chloride (10 mmol) is dissolved in anhydrous acetone (30 mL), added to a solution of ammonium thiocyanate (10 mmol) in dry acetone and refluxed for 1 h. The ammonium chloride is removed by filtration and the suitable substituted aniline (10 mmol) dissolved in anhydrous acetone is added while stirring. The mixture is heated under reflux for 1 h and then poured into ten times its volume of cold water when the N-(1-methyl-1H-pyrazole-4-carbonyl)-N′-(aryl)-thioureas (4a–j) precipitated as solids, or slowly crystallizing oils. The compounds were recrystallized from isopropanol. The characterizations of 4a–b and 4g are presented in previous papers.

4.1.1.1. N-[(1-methyl-1H-pyrazole-4-yl)carbonyl]-N′-(2-methoxyphenyl)-thiourea (4c). Yield 65%, mp 180–181 °C. IR (cm^{-1}): 1659 (C=O). ^{1}H NMR (DMSO-d_6, ppm): 12.30 (s, 1H, –NH–CS–), 11.28 (s, 1H, NH), 8.60 (s, 1H, –N–N=CH–), 8.53 (dd, J = 8.1 Hz, J = 1.5 Hz, 1H, ArH), 8.21 (s, 1H, = N–N–CH–), 7.22 (td, J = 8.1 Hz, J = 1.5 Hz,

1H, ArH), 7.12 (dd, $J = 8.1$ Hz, $J = 1.5$ Hz, 1H, ArH), 6.98 (td, $J = 8.1$ Hz, $J = 1.5$ Hz, 1H, ArH), 3.89 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, ppm): 178.32, 163.07, 150.73, 140.47, 134.67, 126.96, 126.65, 123.43, 119.80, 115.52, 111.36, 56.06, 39.11. Calcd. for $C_{13}H_{14}N_4O_2S$: C, 53.78; H, 4.86; N, 19.30; S, 11.04. Found: C, 53.44; H, 4.99; N, 19.72; S, 11.06%.

4.1.1.2. N-[(1-methyl-1H-pyrazole-4-yl)carbonyl]-N′-(3-methoxyphenyl)-thiourea (4d). Yield 67%, mp 164–165 °C. IR (cm^{-1}): 1665 (C=O). ^{1}H NMR (DMSO-d_6, ppm): 12.50 (s, 1H, –NH–CS–), 11.37 (s, 1H, NH), 8.60 (s, 1H, –N–N=CH–), 8.21 (s, 1H, = N–N–CH–), 7.43 (t, $J = 2.1$ Hz, 1H, ArH), 7.31 (t, $J = 8.1$ Hz, 1H, ArH), 7.18 (dd, $J = 8.1$ Hz, $J = 2.1$ Hz, 1H, ArH), 6.83 (ddd, $J = 8.1$ Hz, $J = 2.1$ Hz, $J = 0.8$ Hz, 1H, ArH), 3.89 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, ppm): 179.69, 163.74, 159.94, 141.01, 139.66, 135.21, 130.10, 116.88, 116.13, 112.41, 110.32, 55.85, 39.68. Calcd. for $C_{13}H_{14}N_4O_2S$: C, 53.78; H, 4.86; N, 19.30; S, 11.04. Found: C, 53.52; H, 5.04; N, 19.65; S, 10.94%.

4.1.1.3. N-[(1-methyl-1H-pyrazole-4-yl)carbonyl]-N′-(4-methoxyphenyl)-thiourea (4e). Yield 73%, mp 192–193 °C. IR (cm^{-1}): 1666 (C=O). ^{1}H NMR (DMSO-d_6, ppm): 12.50 (s, 1H, –NH–CS–), 11.27 (s, 1H, –NH–CO–), 8.58 (s, 1H, -N–N=CH-), 8.20 (s, 1H, = N–N–CH–), 7.52 (d, $J = 9.1$ Hz, 2H, ArH), 6.95 (d, $J = 9.1$ Hz, 2H, ArH), 3.89 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, ppm): 180.06, 163.68, 158.02, 140.96, 135.15, 131.46, 126.61, 115.48, 114.40, 55.91, 39.66. Calcd. for $C_{13}H_{14}N_4O_2S$: C, 53.78; H, 4.86; N, 19.30; S, 11.04; Found: C, 53.44; H, 4.87; N, 19.58; S, 10.96%.

4.1.1.4. N-[(1-methyl-1H-pyrazole-4-yl)carbonyl]-N′-(3-chlorophenyl)-thiourea (4f). Yield 72%, mp 168–169 °C. IR (cm^{-1}): 1670 (C=O). ^{1}H NMR (DMSO-d_6, ppm): 12.50 (bs, 1H, –NH–CS–), 11.20 (bs, 1H, –NH–CO–), 8.59 (s, 1H, –N–N=CH–), 8.20 (s, 1H, = N–N–CH–), 7.93 (t, $J = 2.0$ Hz, 1H, ArH), 7.53 (ddd, $J = 7.9$ Hz, $J = 2.0$ Hz, $J = 1.3$ Hz, 1H, ArH), 7.43 (t, $J = 7.9$ Hz, 1H, ArH), 7.32 (ddd, $J = 7.9$ Hz, $J = 2.0$ Hz, $J = 1.3$ Hz, 1H, ArH), 3.89 Hz (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, ppm): 180.13, 163.56, 141.02, 139.99, 135.26, 133.30, 130.91, 126.68, 124.64, 123.72, 116.07, 39.68. Calcd. for $C_{12}H_{11}ClN_4OS$: C, 48.90; H, 3.76; N, 19.01; S, 10.88; Found: C, 48.77; H, 3.85; N, 18.89; S, 10.95%.

4.1.1.5. N-[(1-methyl-1H-pyrazole-4-yl)carbonyl]-N′-(2,4-dichlorophenyl)-thiourea (4h). Yield 72%, mp 189–190 °C. IR (cm^{-1}): 1668 (C=O). ^{1}H NMR (DMSO-d_6, ppm): 12.50 (bs, 1H, –NH–CS–), 11.20 (bs, 1H, –NH–CO–), 8.60 (s, 1H, –N–N=CH–), 8.22 (s, 1H, = N–N–CH–), 8.05 (d, $J = 8.8$ Hz, 1H, ArH), 7.74 (d, $J = 2.4$ Hz, 1H, ArH), 7.48 (dd, $J = 8.8$ Hz, $J = 2.4$ Hz, 1H, ArH), 3.89 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, ppm): 181.09, 163.74, 141.11, 135.38, 135.24, 131.96, 130.24, 129.83, 129.55, 127.99, 115.89, 39.70. Calcd. for $C_{12}H_{10}Cl_2N_4OS$: C, 43.78; H, 3.06; N, 17.02; S, 9.74; Found: C, 43.87; H, 3.21; N, 17.11; S, 9.61%.

4.1.1.6. N-[(1-methyl-1H-pyrazole-4-yl)carbonyl]-N′-(2,5-dichlorophenyl)-thiourea (4i). Yield 70%, mp 191–193 °C. IR (cm^{-1}): 1672 (C=O). ^{1}H NMR (DMSO-d_6, ppm): 12.50 (bs, 1H, –NH–CS–), 11.20 (bs, 1H, –NH–CO–), 8.59 (s, 1H, –N–N=CH–), 8.28 (d, $J = 2.6$ Hz, 1H, ArH), 8.23 (s, 1H, = N–N–CH–), 7.62 (d, $J = 8.5$ Hz, 1H, ArH), 7.39 (dd, $J = 8.5$ Hz, $J = 2.6$ Hz, 1H, ArH), 3.90 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, ppm): 180.91, 163.76, 141.14, 137.21, 135.43, 131.74, 131.42, 128.28, 127.74, 127.43, 115.86, 39.71. Calcd. for $C_{12}H_{10}Cl_2N_4OS$: C, 43.78; H, 3.06; N, 17.02; S, 9.74; Found: C, 43.69; H, 2.97; N, 17.30; S, 9.81%.

4.1.1.7. N-[(1-methyl-1H-pyrazole-4-yl)carbonyl]-N′-(2,6-dichlorophenyl)-thiourea (4j). Yield 66%, mp 186–187 °C. IR (cm^{-1}): 1666 (C=O). ^{1}H NMR (DMSO-d_6, ppm): 12.21 (bs, 1H, –NH–CS–), 11.60 (bs, 1H, –NH–CO–), 8.59 (s, 1H, –N–N=CH–), 8.21 (s, 1H, = N–N–CH–), 7.56 (d, $J = 8.1$ Hz, 2H, ArH), 7.39 (t, $J = 8.1$ Hz, 1H, ArH), 3.90 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, ppm): 180.88, 163.47, 141.05, 135.33, 134.83, 134.39, 130.40, 129.13, 115.99, 39.70. Calcd. for $C_{12}H_{10}Cl_2N_4OS$: C, 43.78; H, 3.06; N, 17.02; S, 9.74; Found: C, 43.84; H, 2.97; N, 16.92; S, 9.88%.

4.1.2. Synthesis procedure for compound 5

The 1-methyl-1H-pyrazole-4-carbonyl chloride (10 mmol) is dissolved in anhydrous acetonitrile (30 mL), added to a solution of ammonium thiocyanate (0.76 g, 10 mmol) and refluxed for 1 h. The ammonium chloride is removed by filtration and an acetonitrile solution of phenylhydrazine (10 mmol) is added. The mixture is heated under reflux for 1 h and then poured into ten times its volume of cold water. The compound was recrystallized from isopropanol.

4.1.2.1. 5-(1-Methyl-1H-pyrazol-4-yl)-1-phenyl-1,2-dihydro-1,2,4-triazole-3-thione (5). Yield 65%, mp 228–230 °C ^{1}H NMR (DMSO-d_6, ppm): 8.32 (s, 1H, –N–N=CH–), 8.00 (s, 1H, = N–N–CH–), 7.99 (bd, 2H, ArH), 7.53 (tt, $J = 7.4$ Hz, $J = 1.5$ Hz, 2H, ArH), 7.41 (tt, $J = 7.4$ Hz, $J = 1.5$ Hz, 1H, ArH), 3.93 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, ppm): 165.08, 144.42, 137.75, 137.32, 130.42, 128.70, 127.71, 123.79, 107.24, 39.01. Calcd. for $C_{12}H_{11}N_5S$: C, 56.01; H, 4.31; N, 27.22; S, 12.46; Found: C, 55.88; H, 4.36; N, 27.03; S, 12.55%.

4.1.3. General synthesis procedure for compounds 6a–b

To a solution of N-(1-methyl-1H-pyrazole-4-carbonyl)-N′-(aryl)-thioureas (5 mmol) and triethylamine (15 mmol) in acetone we added, while stirring, a solution of bromoacetone, prepared *in situ*, in the dropping funnel, from bromine (5 mmol) and acetone. The reaction mixture is stirred for 2 h, the solvent is removed by distillation and the solid is washed with water. The compounds were recrystallized from ethyl acetate.

4.1.3.1. 1-Methyl-N-(4-methyl-3-phenylthiazol-2(3H)-ylidene)-1H-pyrazole-4-carboxamide (6a). Yield 63%, mp 167–168 °C. IR (cm^{-1}): 1616 (C=O). ^{1}H NMR (DMSO-d_6, ppm): 7.78 (s, 1H, –N–N=CH–), 7.64–7.56 (m, 3H, ArH), 7.48 (s, 1H, = N–N–CH–), 7.45–7.42 (m, 2H, ArH), 6.75 (s, 1H, = CH), 3.78 (s, 3H, CH_3), 1.98 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, ppm): 169.31, 168.45, 139.92, 137.14, 134.29, 132.78, 129.42, 128.31, 122.16, 117.95, 104.18, 38.75, 14.61. Calcd. for $C_{15}H_{14}N_4OS$: C, 60.38; H, 4.73; N, 18.78; S, 10.75; Found: C, 60.49; H, 4.69; N, 18.83; S, 10.66%.

4.1.3.2. N-(3-(4-chlorophenyl)-4-methylthiazol-2(3H)-ylidene)-1-methyl-1H-pyrazole-4-carboxamide (6b). Yield 65%, mp 238 °C. IR (cm^{-1}): 1587 (C=O). ^{1}H NMR (DMSO-d_6, ppm): 7.79 (s, 1H, –N–N=CH–), 7.56 (d, $J = 8.6$ Hz, 2H, ArH), 7.49 (s, 1H, = N–N–CH–), 7.37 (d, $J = 8.6$ Hz, 2H), 6.81 (s, 1H, = CH), 3.79 (s, 3H, CH_3), 1.38 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, ppm): 171.18, 170.26, 140.19, 137.06, 133.29, 132.21, 130.99, 128.61, 121.75, 119.07, 90.61, 38.68, 25.98. Calcd. for $C_{15}H_{13}ClN_4OS$: C, 54.13; H, 3.94; N, 16.83; S, 9.63; Found: C, 54.22; H, 4.08; N, 16.77; S, 9.60%.

4.1.4. General synthesis procedure for compounds 7a–c

A solution of benzoyl chloride (10 mmol) in anhydrous acetone (30 mL) is added to a solution of ammonium thiocyanate (10 mmol) and refluxed for 1 h. The ammonium chloride is removed by filtration and the suitable pyrazole amine (10 mmol) is added while stirring. The mixture is heated under reflux for 1 h and then poured into ten times its volume of cold water. The compounds were recrystallized from isopropanol.

4.1.4.1. N-Benzoyl-N′-(5-methyl-1H-pyrazol-3-yl)thiourea (7a). Yield 79%, mp 207–209 °C. IR (cm^{-1}): 1662 (C=O). ^{1}H NMR (DMSO-d_6, ppm): 13.10 (s, 1H, = N–NH–), 12.47 (s, 1H, –CS–NH–),

11.56 (s, 1H, –CO–NH–), 7.97 (d, J = 7.4 Hz, 2H, ArH), 7.66 (t, J = 7.4 Hz, 1H, ArH), 7.53 (t, J = 7.4 Hz, 2H, ArH), 6.87 (s, 1H, PyrzH), 2.25 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, ppm): 176.51, 168.68, 146.84, 138.36, 133.17, 132.07, 128.72, 128.43, 97.37, 10.71. Calcd. for $C_{12}H_{12}N_4OS$: C, 55.37; H, 4.65; N, 21.52; S, 12.32; Found: C, 55.44; H, 4.87; N, 21.40; S, 12.21%.

4.1.4.2. N-Benzoyl-N′-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-thiourea (7b). Yield 76%, mp 166–168 °C. IR (cm^{-1}): 1672 (C=O). ^{1}H NMR (DMSO-d_6, ppm): 12.46 (s, 1H, –CS–NH–), 11.80 (s, 1H, –CO–NH–), 7.91 (d, J = 7.4 Hz, 2H, ArH), 7.65 (tt, J = 7.4 Hz, J = 2.2 Hz, 1H, ArH), 7.55 (tt, J = 7.2 Hz, J = 2.2 Hz, 2H, ArH), 7.51 (dt, J = 7.1 Hz, J = 2.3 Hz, 2H, ArH), 7.51 (tt, J = 7.1 Hz, J = 2.3 Hz, 2H, ArH), 7.38 (tt, J = 7.1 Hz, J = 2.3 Hz, 1H, ArH), 6.58 (s, 1H, PyrzH), 2.26 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, ppm): 180.24, 168.02, 147.82, 138.23, 136.74, 133.33, 131.72, 129.20, 128.78, 128.46, 127.58, 123.84, 102.87, 13.87. Calcd. for $C_{18}H_{16}N_4OS$: C, 64.27; H, 4.79; N, 16.65; S, 9.53; Found: C, 64.21; H, 4.81; N, 16.77; S, 9.51%.

4.1.4.3. N-Benzoyl-N′-(3-(4-bromophenyl)-1H-pyrazol-5-yl)-thiourea (7c). Yield 73%, mp 223–224 °C. IR (cm^{-1}): 1665 (C=O). ^{1}H NMR (DMSO-d_6, ppm): 13.37 (s, 1H, NH), 13.17 (s, 1H, –CS–NH–), 11.65 (s, 1H, –CO–NH–), 7.99 (dd, J = 7.2 Hz, J = 1.5 Hz, 2H, ArH), 7.73–7.67 (m, 4H, ArH), 7.67 (tt, J = 7.3 Hz, J = 1.4 Hz, 1H, ArH), 7.54 (bt, 2H, ArH), 7.52 (s, 1H, PyrzH). ^{13}C NMR (DMSO-d_6, ppm): 177.07, 168.75, 147.79, 140.77, 133.27, 132.10, 128.79, 128.49, 128.20, 128.13, 127.16, 121.58, 96.08. Calcd. for $C_{17}H_{13}BrN_4OS$: C, 50.88; H, 3.27; N, 13.96; S, 7.99; Found: C, 51.03; H, 3.28; N, 13.88; S, 8.11%.

4.2. Cytotoxic assay on human malignant cells

The anticancer activity was performed according to the *in vitro* cancer screen methodology of the National Cancer Institute, Bethesda, USA [20–22]. The compounds were added to the cell culture as 10^{-5} M solution in dimethyl sulfoxide and the cells were incubated for 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. The results for each tested compounds are reported as growth percentages compared with the untreated control cells. The cellular growth is measured spectrophotometrically at a wavelength of 515 nm using sulforhodamine B. For the most active compounds a 5-dose assay was performed using 10-fold serial dilutions of five drug concentrations raging from 10^{-8} to 10^{-4} M. Using the seven absorbance measurements: time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti), the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

$[(Ti - Tz)/(C - Tz)] \times 100$ for concentrations for which $Ti \geq Tz$.

$[(Ti - Tz)/Tz] \times 100$ for concentrations for which $Ti < Tz$.

Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50% (GI_{50}) is calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by sulforhodamine B staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from $Ti = Tz$. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(Ti - Tz)/Tz] \times 100 = -50$. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested.

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