The use of pyridazine thione derivative in the preparation of some new heterocyclic compounds with expected antitumor activity

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**ABSTRACT**

Pyridazine thione 1 was used as a key intermediate for the preparation of numerous pyridazine derivatives. Reaction of 1 with copper bronze, nitrous acid, anthranilic acid, acetylacetone and benzalacetophenone afforded the bis pyridazine, quinazolinone, diketo and the adduct derivatives. Condensation of the diketo and the adduct derivatives with hydrazine hydrate gave the corresponding pyrazole and hydrazone derivatives. The behaviour of 1 towards thiourea, ethyl chloroacetate and aromatic amines has also been studied. The reaction of the resulting products with diethyl malonate/acetyl chloride, aromatic amines, sodium hydroxide and hydrazine hydrate has also been taken into consideration. The antitumor activity of some of the synthesized compounds were tested.

**Keywords:** pyridazine and antitumor activity

**INTRODUCTION**

In the last several decades, pyridazine and indole derivatives have received considerable attention due to their wide-range applications. Pyridazines are reported to exhibit antibacterial\(^1\), antifungal\(^1\), antituberculosis\(^2\), antinociceptive\(^3\), anthelmintic\(^4\), antidiabetic\(^5\) activities and also as human rhinovirus (HRV-3) inhibitors\(^6\). On the other hand, indole derivatives exhibit antioxidant\(^7\), antifungal\(^8\), antitumor\(^9\), anti-hepatitis C virus\(^10\) activities.

Encouraged by these reports, we thought of synthesizing a new series of pyridazines containing the 2-phenylindole at 4-position hoping to improve the antitumor activity of the new compounds.

**EXPERIMENTAL SECTION**

All melting points are uncorrected. IR spectra (KBr) were recorded with a Perkin Elmer Spectrum RXIFT-IR systems. \(^1\)HNMR were measured with a Varian Gemini 200 MHz instrument using TMS as internal standard and mass spectra were measured with a Shimadzu GC-MS-QP 100 EX mass spectrometer.

**Synthesis of 3,3’-(6,6-bis(3,4-dimethylphenyl)-3,3’-bipyridazine-4,4’-diyl)-bis (2-phenyl-1H-indole) (2)**

A mixture of 1 (0.01 mol), xylene (30 mL) and copper bronze (2g) was refluxed for 6h, concentrated, cooled, the solid product separated was filtered off and recrystallized from ethanol to give 2 (m.p 200°C). Analysis for C\(_{25}\)H\(_{42}\)N\(_6\)(%): Calcd. C 83.17, H 5.64, N 11.19; found C 83.22, H, 5.62, N11.16.
Synthesis of 1,2-bis(6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl) pyridazin-3-yl) disulfane (3).
To a solution of 1 (0.01 mol) in ethanol (20 ml), sodium nitrite (0.01 mol) and acetic acid (3 ml) were added. The mixture was stirred at room temperature for 4 h. The solid product which separated out was crystallized from ethanol to give 3 (m.p. 273°C). Analysis for C52H40N6S2 (%): Calcd. C 76.82, H 4.96, N 10.34, S 7.89; found C 76.80, H 4.98, N 10.32, S 7.91.

Synthesis of 2-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl)-10H-pyridazino [6,1-b] quinazolin-10-one (4a), 8-bromo-2-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl)-10H-pyridazin[6,1-b] quinazolin-10-one (4b), 3-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl) pyridazin-3(2H)-ylidene) pentane-2,4-dione (5) and 3-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl)pyridazin-3-ylthio)-1,3-diphenylpropan-1-one (7).
Solution of 1 (0.01 mol), anthranilic acid, 5-bromoanthranilic acid, acetylacetone or benzalacetophenone (0.01 mol) in ethanol or butanol (30 ml) was refluxed for 6-7 h. The solid separated on cooling was crystallized from ethanol (4a m.p. 212°C, 4b m.p. 211°C, 5m.p. 189°C, 7 m.p. 194°C. Analysis for C33H24N4O (%): Calcd. C 80.47, H 4.91, N 11.37; found C 80.43, H 4.90, N 11.41; for C33H23BrN4O (%): Calcd. C 69.36, H 4.06, Br 13.98, N 9.80; found C 69.38, H 4.07, Br 13.97, N 9.82; for C31H27N3O2 (%): Calcd. C 78.62, H 5.75, N 8.87; found C 78.58, H 5.77, N 8.89; for C41H33N5OS (%): Calcd. C 79.79, H 5.40, N 6.82, S 5.21; found C 79.95, H 5.41, N 6.85, S 5.19.

Synthesis of 3-(3-(3,5-dimethyl-4H-pyrazol-4-ylidene) – 6 - (3,4-dimethylphenyl)-2,3-dihydropyridazin-4-yl)-2-phenyl-1H-indole (6), 3-(6-(3,4-dimethylphenyl)-3-(3-hydrazono-1,3-diphenylpropylthio) pyridazin-4-yl)-2-phenyl-1H-indole (8) and 2-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol -3-yl) pyridazin-3-ylthio) aceto hydrazide (14).
To a solution of 5,7 or 11 (0.01 mol) in ethanol (20 ml), hydrazine hydrate (0.01 mol) was added and the reaction mixture was refluxed for 4-6 h. The solid separated on cooling was crystallized from ethanol (6 m.p. 203°C, 8 m.p. 170°C, 14 m.p. 178°C. Analysis for C31H25N5 (%): Calcd. C 79.29, H 5.80, N 14.91; found C 79.25, H 5.82, N 14.93; for C28H25N5S (%): Calcd. 78.19, H 5.60, N 11.12, S 5.09; found C 78.21, H 5.58, N 11.10, S 5.11; for C29H32N5OS (%): Calcd. C 70.12, H 5.25, N 14.60, S 6.69; found C 70.02, H 5.23, N 14.62, S 6.79.
Synthesis of 1-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl) pyridazin-3-yl) thiourea (9). Solution of 1 (0.01 mol) and thiourea (0.01 mol) in dimethyl formamide (30 ml) was refluxed for 16 h. The solid separated on cooling was crystallized from ethanol to give 9 (m.p 173°C). Analysis for C_{27}H_{23}N_{5}S(%) : Calcd. C 72.13, H 5.16, N 15.58, S 7.13; found C 72.10, H 5.14, N 15.60, S 7.16.

Synthesis of 1-acetyl-3-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl) pyridazin-3-yl)-2-thioxodihydropyrimidine-4-,6- (1H, 5H)-dione (10). Solution of 9 (0.01 mol), malonic acid (0.01 mol) and acetyl chloride (5 ml) in ethanol (20 ml) was refluxed for 2 h. The solid separated on cooling was crystallized from ethanol to give 10 (m.p. 190°C). Analysis for C_{32}H_{25}N_{5}O_{3}S(%) : Calcd. C 68.68, H 4.50, N 12.51, S 5.73; found: C 68.70, H 4.51, N 12.50, S 5.71.

Synthesis of ethyl 2-(6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl) pyridazin-3-ylthio) acetate (11). A mixture of 1 (0.01 mol), anhydrous potassium carbonate (0.03 mol), ethyl chloroacetate (0.03 mol) and dry acetone (50 ml) was refluxed for 24 h. on a water bath. The solid product obtained after hot filtration and evaporation of the solvent was crystallized from ethanol to give 11 (m.p. 141°C). Analysis for C_{30}H_{27}N_{3}O_{2}S(%) : Calcd. C 73.00, H 5.51, N 8.51. S 6.50; Found: C 72.80, H 5.71, N 8.49, S 6.52.

Synthesis of 2- (6- (3,4-dimethylphenyl) -4- (2-phenyl-1H-indol-3-yl) pyridazin-3-ylthio) -N- (naphthalen-1-yl) acetamide (12) , N-benzyl -6-(3,4-dimethylphenyl) -4 - (2-phenyl-1H-indol-3-yl) pyridazine -3-amine (17a) and 6-(3,4-dimethylphenyl) -4 - (2-phenyl-1H-indol-3-yl) – N - (pyridin-2-yl) pyridazin-3-amine (17b). Solution of 11 or 1 (0.01 mol), α-naphthylamine, benzylamine or 2-aminopyridine (0.01 mol) in ethanol (30 ml) was refluxed for 6 h. The solid separated on cooling was crystallized from ethanol (12 m.p. 197°C, 17a m.p. 150°C, 17b m.p. 132°C). Analysis for C_{38}H_{30}N_{4}OS(%) : Calcd C 77.26, H 5.12, N 9.48, S 5.43; Found C77.23, H 5.15, N 9.45,
Synthesis of 3-(6-(3,4-dimethylphenyl)-3-(methylthio) pyridazin-4-yl)-2-phenyl -1H-indole (13).
To a 10% ethanolic sodium hydroxide solution (50 ml), compound 11 was added and the reaction mixture was refluxed for 6h. The solid separated after concentration and cooling was washed well with water and crystallized from ethanol to give 13 (m.p. 160° C). Analysis for C_{27}H_{23}N_{3}S(%) : Calcd. C 76.93, H 5.50, N 9.97, S 7.61; Found C 76.90, H 5.57, N 9.90, S 7.64.

Synthesis of 5-(6-(3,4-dimethyl phenyl)-4-(2-phenyl-1H-indol-3-yl) pyridazin-3-ylthio)methyl)-1,3,4-oxadiazole-2 (3H)- thione (15).
To a suspension of 14 (0.01 mol) in ethanol (15 ml), CS_{2} (5ml) and KOH (0.005 mol) were added. The reaction mixture was refluxed for 2h. on a water bath, cooled then poured onto ice and acidified with dil. HCl. The solid obtained was crystallized from ethanol to give 15 (m.p. 216° C). Analysis for C_{29}H_{23}N_{5}OS_{2}(%) : Calcd. C 66.77, H 4.44, N 13.43, S 12.29; found C 66.73, H 4.47, N 13.47, S 12.25.

Synthesis of 4- (6- (3,4-dimethylphenyl) -4- (2-phenyl-1H-indol-3-yl) pyridazin-3-ylthio)-5-(2-hydroxyphenyl)-1,2-dihydro-3-H-pyrazol-3-one (16).
To a solution of 14(0.01 mol) in ethanol (30 ml), salicylaldehyde was added dropwise while stirring at room temperature. Stirring was continued for 2h. The orange crystals formed was collected by filtration and recrystallized from ethanol to give 16 (m.p. 200° C). Analysis for C_{35}H_{27}N_{5}O_{2}S(%) : Calcd. C 72.27, H 4.68, N 12.04, S 5.51; Found C 72.17, H 4.62, N 5.57, S 5.41.

Sulforhodamine-B(SRB) assay of cytotoxic activity.
MCF7 (breast carcinoma cell line), HEPG2 (hepatocellular carcinoma cell line), HCT 116 (colon carcinoma cell line) were obtained frozen in liquid nitrogen (-180° C) from the American type culture collection. The tumor cell line were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing. Potential cytotoxicity of 4a-b, 5,7,13, 17a and 17b. were tested using method of Skehan et al. (11).

Principle
The sensitivity of the human tumor cell lines to thymoquinone was determined by the SRB assay. SRB is a brought pink aminoxanthrene dye with two sulfonic groups. It is a protein stain that binds to the amino group of intracellular proteins under mildly acidic conditions to proceed a sensitive index of cellular protein content.

Procedure
1. Cells were used when 90% confluence was reached in T25 flasks. Adherent cell lines were harvested with 0.025% trypsin. Viability was determined by trypan blue exclusion using the inverted microscope (Olympus 1 x 70, Tokyo, Japan).
2. Cells were seeded in 96- well microtiter plates at a concentration of 5 x 10^{4} – 10^{5} cell/well in a fresh medium and left to attach to the plates for 24h.
3. After 24h, cells were incubated with the appropriate concentration ranges of drugs, completed to total of 200 µl volume/well using fresh medium and incubation was continued for 24, 48 and 72h. Control cells were treated with vehicle alone. For each drug concentration, 4 wells were used.
4. Following 24, 48 and 72h, treatment, the cells were fixed with 50µl cold 50% trichloroacetic acid for 1h. at 4°C.
5. Wells were washed 5 times with distilled water and stained for 30 min. at room temperature with 50 µl 0.4% SRB dissolved in 1% acetic acid.
6. The wells were then washed 4 times with 1% acetic acid.
7. The plates were air-dried and the dye was solubilized with 100µl/well or 10 mM tris base (Ph 10.5) for 5 min.
8. The optical density (O.D) of each well was measured spectrophotometrically at 564 nm with an ELIZA microplate reader (Meter tech £960, U.S.A). The mean background absorbance was automatically subtracted and mean values of each drug concentration was calculated. The relation between survival fraction and compound concentration was plotted to get the survival curve of each tumor cell lines. (Fig. 1) .The IC 50 values (the concentration of thymoquinone required to produce 50% inhibition of cell growth (Fig. 2).

RESULTS AND DISCUSSION

The new derivatives were prepared following the reaction sequences depicted in Schemes 1 and 2.
Treatment of 6-(3,4-dimethylphenyl)-4-(2-phenyl-1H-indol-3-yl) pyridazine-3(2H)-thione\textsuperscript{(12)} (1) with copper bronze in boiling xylene yielded the bipyridazine derivative (2). Its IR spectrum was devoid of \textit{\nu}C=\textit{S} and showed the C=N absorption band at 1647 and NH 3397 cm\textsuperscript{-1}. Its mass spectrum showed an ion peak at m/z 751 (M+1) (0.27\%).

On the other hand, compound 1 was oxidized to the disulfane derivative 3 upon treatment with sodium nitrite/acetic acid mixture. Its IR spectrum showed the band of C=S and showed the C=N absorptions at 1606 and 1595 cm\textsuperscript{-1}. The \textit{\nu}H\textsubscript{2}N\textsubscript{H} of 3 was 3409 cm\textsuperscript{-1}. Its mass spectrum showed the molecular ion peak at m/z 493 (1.05\%).

Reaction of 1 with anthranilic acid and 5-bromoanthranilic acid gave the quinazolinone derivatives 4\textsubscript{a,b} through elimination of one molecule of H\textsubscript{2}S and H\textsubscript{2}O. Their IR spectra exhibited bands for \textit{\nu}C=\textit{O} at 1615, 1674, \textit{\nu}C=S at 1590 and \textit{\nu}C=\textit{N} at 1604, 1595 and \textit{\nu}NH at 3379 and 3371 cm\textsuperscript{-1}. The \textit{\nu}HNMR (DMSO-d\textsubscript{6}) spectrum of 4\textsubscript{a} exhibited signals at 8.35-6.86 (18H, m, ArH), 3.98 (2H, s, CH\textsubscript{2}) and 11.76 (1H, s, NH). The mass spectrum of 4\textsubscript{a} showed an ion peak at m/z 479 (24.67\%).

It was stated that pyrazole derivatives showed anti-cancer activities and as a novel carries of nitric oxide, molluscicidal\textsuperscript{(17)}, anti-inflammatory\textsuperscript{(16)}, antioxidant\textsuperscript{(16)}, antimicrobial\textsuperscript{(16)}, molluscicidal\textsuperscript{(17)}, anti-angiogenic\textsuperscript{(18)}, activities and as a novel carries of nitric oxide\textsuperscript{(19)}. This prompted the authors to synthesize pyrazole derivative through the reaction of the pyridazine thione with acetylacetone to give the diketo compound 5 followed by cyclization with the binucleophile hydrazine hydrate to give the pyrazole derivative 6. The IR spectrum of 5 exhibited bands for \textit{\nu}C=\textit{O} at 1708, \textit{\nu}C=S at 1610 and \textit{\nu}NH at 3400 cm\textsuperscript{-1}. Its mass spectrum showed an ion peak at m/z 474 (M+1) (0.05\%). While the IR spectrum of 6 was devoid of \textit{\nu}C=\textit{O} and showed \textit{\nu}C=S and \textit{\nu}NH at 1582 and 3441 cm\textsuperscript{-1}.

The nucleophilic addition of the pyridazinethione 1 to benzalacetophenone gave the adduct 7. Its IR exhibited bands for \textit{\nu}C=S at 1594, \textit{\nu}C=\textit{O} at 1604 and \textit{\nu}NH at 3432 cm\textsuperscript{-1}. The structure of 7 was further established by its IR spectrum showing the bands at 1604-1616, \textit{\nu}C=\textit{N} at 1604, and \textit{\nu}NH at 3441 cm\textsuperscript{-1}. Its mass spectrum showed the ion peak at m/z 630 (M+1) (6.23\%).

Treatment of compound 1 with thiourea in boiling DMF gave the thiourea derivative 9 through the nucleophilic attack of the nitrogen of thiourea to the carbon of the thione moiety followed by elimination of one molecule of H\textsubscript{2}S. Its IR spectrum exhibited absorption bands at 1594, 1402, 3373, 3261, 3162 cm\textsuperscript{-1} for \textit{\nu}C=S, \textit{\nu}C=\textit{O} and \textit{\nu}NH at 3403, 3213 and 3117 cm\textsuperscript{-1}. The \textit{\nu}HNMR (DMSO-d\textsubscript{6}) and 11.76 (1H, s, NH). The mass spectrum of 9 showed a molecular ion peak at m/z 479 (24.67\%).

The thiodihydroxyprimidinedione (10) can be prepared through the one - pot reaction of compound 9, malonic acid and acetyl chloride. Its IR spectrum exhibited bands at 1714, 1637, 1590, 1400 and 3413 for \textit{\nu}C=\textit{O}, \textit{\nu}C=S, \textit{\nu}C=\textit{N}, \textit{\nu}C=S and \textit{\nu}NH.

The present investigation also deals with carboethoxyxymethylation of the thiopyridazine 1 through its treatment with ethyl chloroacetate in dry acetone in the presence of potassium carbonate to give compound 11. Its IR spectrum showed the characteristic for \textit{\nu}C=\textit{O} (ester) at 1729, \textit{\nu}C=\textit{S} at 1604 and \textit{\nu}NH at 3368 cm\textsuperscript{-1}, while its mass spectrum showed the molecular ion peak at m/z 493 (1.05). The resulting ester 11 has been used as starting material for the preparation of a series of new compounds.

Reaction of 11 with \textit{a}-naphthylamine gave the acetamide derivative 12. Its IR spectrum showed \textit{\nu}C=\textit{O} at 1672, \textit{\nu}C=S at 1606 and \textit{\nu}NH at 3409 cm\textsuperscript{-1}, while its mass spectrum showed an ion peak at m/z 589 (M–1) (4.36\%).

Alkaline hydrolysis of compound 11 using ethanolic sodium hydroxide solution afforded the S-alkylated product 13 through decarboethoxylation. Its IR spectrum was devoid of \textit{\nu}C=\textit{O} and showed \textit{\nu}C=S at 1599 and \textit{\nu}NH at 3434 cm\textsuperscript{-1}, while its \textit{\nu}HNMR (DMSO-d\textsubscript{6}) spectrum exhibited signals at 7.84-6.87 (13H, m, Ar-H), 2.32 (6H, s, 2 x CH\textsubscript{2}) 2.13 (3H, s, SCH\textsubscript{3}) and 11.53 (1H, s, NH). Its mass spectrum showed the molecular ion peak at m/z 421 (4.36\%).

On the other hand, reaction of 11 with hydrazine hydrate afforded the corresponding hydrazide derivative 14, which can be used for the preparation of the oxadiazole derivative 15 and the pyrazole derivative 16, through its reaction with carbon disulfide/ KOH and/or salicylaldehyde. The IR spectrum of 11 showed \textit{\nu}C=\textit{O} at 1658, \textit{\nu}C=S at 1598 and \textit{\nu}NH at 3403, 3213 and 3117 cm\textsuperscript{-1}, its mass spectrum showed the molecular ion peak at m/z 479 (24.67\%). While the IR spectrum of 14 showed \textit{\nu}C=S at 1618, \textit{\nu}C=S at 1604 and \textit{\nu}NH at 3441.

Reaction of compound 1 with benzylamine and 2- aminopyridine yielded Schiff bases 17\textsubscript{a} and 17\textsubscript{b}, respectively. Their IR spectra were devoid of \textit{\nu}C=S and showed \textit{\nu}C=N at 1604-1616, \textit{\nu}NH at 3404, 3426 cm\textsuperscript{-1}(broad). The \textit{\nu}HNMR of 17\textsubscript{a} exhibited signals at 8.35- 6.86 (18H, m, ArH), 3.98 (2H, s, CH\textsubscript{2}), 2.25 (6H, s, 2 x CH\textsubscript{3}) and 11.37
(2H, s, 2 x NH), while that of 17b exhibited signals at 7.83-6.50 (17H, m, Ar–H), 2.47 (6H, s, 2XCH₃) and 11.51 (2H, s, 2 xNH). The mass spectra of 17a showed the molecular ion peak at m/z 480 (6.14%), while that of 17b at m/z 467 (17.74%).

Cytotoxicity against different human cancer cell lines in vitro for evaluation of anti-tumor cytotoxicity of compounds 4a, 4b, 5, 7, 13, 17a and 17b, three different human cancer cell lines were used: MCF7 (breast carcinoma cell line), HEPG2 (hepatocellular carcinoma cell line), HCT116 (colon carcinoma cell line) cytotoxicity and IC₅₀ values of the tested compounds are shown in Fig. 1 and 2. The survival fractions were gradually decreased as the concentration of the tested compounds were increased (Table 1).

From Fig. 1, it has been shown that 5, 7,13,17a and 17b are the compounds of lowest IC₅₀ which means that they are the most effective cytotoxic drugs, accordingly compounds 13, 17a and 17b can be used as very potent cytotoxic drug for colon carcinoma cell, while 5 and 7 as moderate cytotoxic drug for colon and liver carcinoma cell respectively, while the remaining compounds are very weak cytotoxic drug.

Fig. (1): Effect of some new prepared compounds on different tumor cells as cytotoxic drug
Fig. (2): 0-10 Very potent cytotoxic drug, 10-20 Moderate cytotoxic drug> 20 Very weak cytotoxic drug.

Table (1): Effect of some new prepared compounds on different tumor cells as cytotoxic drug.

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CONCLUSION

A series of 4a, 4b, 5, 7, 13, 17a and 17b compounds have different anti-tumor effects and IC$_{50}$ values of them were discussed. Compounds 13, 17a and 17b can be used as very potent cytotoxic drug for colon carcinoma cell, while 5 and 7 as moderate cytotoxic drug for colon and liver carcinoma cell respectively, while the remaining compounds are very weak cytotoxic drug.

REFERENCES