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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and preliminary evaluation activity studies of novel 4-(aryl/heteroaryl-2-ylmethyl)-6-phenyl-2-[3-(4-substituted-piperazine-1-yl)propyl]pyridazin-3(*2H*)-one derivatives as anticancer agents

M. S. R. Murty · B. Ramalingeswara Rao · Kesur R. Ram · J. S. Yadav · Jayesh Antony · Ruby John Anto

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Abstract A series of new 4-(aryl/heteroaryl-2-ylmethyl)-6-phenyl-2-[3-(4-substituted piperazine-1-yl)propyl] pyridazin-3(*2H*)-one derivatives were synthesized. The structures of the compounds were confirmed by IR, ^{1}H NMR, and mass spectral data. All the compounds were evaluated for their cytotoxicity toward five human cancer cell lines of different origins viz; HeLa (Cervical), SKBR3 (Breast), HCT116 (Colon), A375 (Skin) & H1299 (Lung) at different concentrations and the IC_{50} values were determined. HCT116 and HeLa are the most sensitive against the compounds studied. One of them displayed moderate cytotoxicity against SKBR3. Majority of the compounds exhibited good to moderate activity.

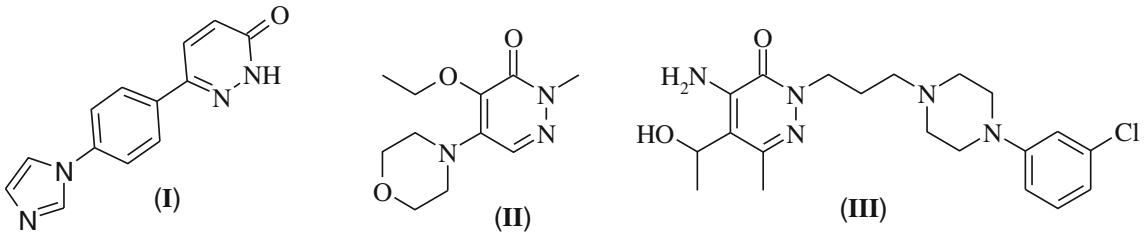
Keywords Pyridazinones · Cyclic amines · Hydrazide · Anticancer activity

M. S. R. Murty (✉) · B. R. Rao · K. R. Ram · J. S. Yadav
Organic Chemistry Division-I, Discovery Laboratory, Indian Institute of Chemical Technology, Uppal Road, Hyderabad 500607, India
e-mail: msrmurty@ymail.com

J. Antony · R. J. Anto
Integrated Cancer Research Program, Division of Cancer Research, Rajiv Gandhi Centre for Biotechnology, Thycaud PO, Kerala, Thiruvananthapuram 695014, India

Introduction

The discovery that several novel series of 3(*2H*)-pyridazinones possess characteristic pharmacological and biological activities stimulated great interest in pyridazine chemistry, which continues to this day. Thus, the pyridazine and its 3-oxo derivatives, i.e., the pyridazinones have attracted a great deal of attention because of the wide spectrum of their pharmaceutical and agrochemical activities. They are widely recognized as versatile scaffolds with a diverse set of biological activities. Particularly pyridazinone derivatives were found to exhibit various pharmaceutical activities such as analgesic (Asif *et al.*, 2011), anti-inflammatory (Gökçe *et al.*, 2009, Sahin *et al.*, 2004), antidepressant (Coelho *et al.*, 2003), antihypertensive (Demirayak *et al.*, 2004, Anees Siddiqui *et al.*, 2011), antithrombotic (Monge *et al.*, 1987), anticonvulsant (Rubat *et al.*, 1990), cardiotonic (Sircar *et al.*, 1987), diuretics (Akahane *et al.*, 1999), and anti-HIV (Livermone *et al.*, 1993) activities. Certain pyridazinone derivatives containing the 2-phenyl-indolyl moiety have shown anti-tumor activity (Ghaffar *et al.*, 2011). Siddiqui et al. synthesized and evaluated the antinociceptive (Anees Siddiqui *et al.*, 2010), activities of the compounds having 6-(substituted-phenyl)-2-(substitutedmethyl)-4,5-dihydropyridazin-3(2H)-one derivatives. 3(*2H*)-Pyridazinone derivatives have been reported as analgesic and anti-inflammatory agents without gastrointestinal side effect. Imazodan (**I**) is reported to show ionotropic properties and Emorfazone A (**II**) is an analgesic and anti-inflammatory compound marketed as pentoil and nandron. Emorfazone E (**III**) contains pyridazinone skeleton bearing piperazine moiety which is also a pharmacologically active molecule and the structures of the above biomolecules are illustrated below.



A number of [(3-chlorophenyl) piperazinyl propyl] pyridazinone and the corresponding isoxazolo pyridazinones, having the arylpiperazinyl substructure present in the molecule are found to be very potent antinociceptive agents (Giovannoni *et al.*, 2003). Substituted 6-aryl-pyridazin-3(*2H*)-ones were found to exhibit anti cancer activity (Ahmad *et al.*, 2010), against various cell lines. Various piperazine derivatives were found to exhibit different pharmaceutical activities. This motif was found in many drug candidates displaying anticancer (Can-Cheng *et al.*, 2004), anti microbial (Chaudhary *et al.*, 2006) activity. Recent studies reveals that certain triazine substituted piperzines have shown anticancer activity (Patel *et al.*, 2011).

Results and discussion

Based on the above observations and continuing our efforts toward the synthesis of new pharmacologically active heterocyclic compounds with expected biological activities, prompted us to synthesize pyridazin-3 (2*H*)-one derivatives bearing cyclic amine moiety i.e., morpholine or substituted piperazines. The cyclic amine component will be linked to the lactam nitrogen of the pyridazinone ring with a three-carbon chain spacer. Thus, as a part of our program aimed at developing simple and efficient syntheses of pharmacologically useful pyridazinones, we have synthesized a new series of 4-(aryl/heteroaryl-2-ylmethyl)-6-phenyl-2-[3-(4-substituted piperazine-1-yl)propyl]pyridazin-3(*2H*)-one derivatives to evaluate the new compounds for anti cancer activity.

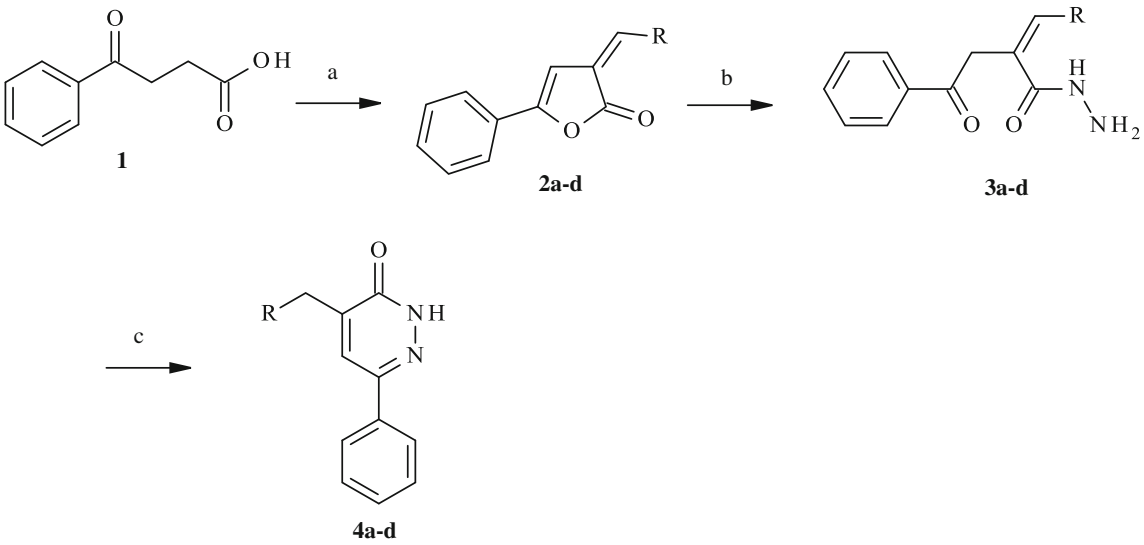
Chemistry

The main idea of synthesizing new piperazine containing pyridazin-3(*2H*)-ones derivatives is to study the cytotoxicity of the products by varying the substituents on pyridazin-3(*2H*)-one moiety as well as on cyclic amine moiety as these additional groups may enhance the biological activity. The title compounds were synthesized from 3-aroyl propionic acid via 2(*3H*)-furanones (**2**) as key intermediate by the following sequence of the reactions. The starting material 3-aroyl propionic acid (**1**) was prepared by condensing benzene with succinic anhydride in presence of anhydrous aluminum chloride by Friedel-Crafts acylation conditions. The compounds 3-substituted-5-phenyl-2-yl-methylene-2-(*3H*)-furanones (**2a–d**) were synthesized from (**1**) by reacting with aromatic aldehydes in the presence of sodium acetate in acetic anhydride. The structures of the furanones (**2a–d**) were confirmed by IR spectrum which show an absorption band at 1765–1787 cm^{-1} for υC=O, characteristic of the lactone carbonyl group. The 2-(*3H*)-furanones (**2a–d**) were reacted with hydrazine hydrate in ethanol at 10–15°C to give the corresponding 3-aroyl-2-(substituted-4-yl-methylene)-propionic acid hydrazide **3a–d**. The presence of IR absorption band at 3110–3340 cm^{-1} (broad band) characteristic of the NH group, and bands at 1650–1665 cm^{-1} and 1678–1700 cm^{-1}, characteristic of the amide carbonyl and ketone groups, respectively. The hydrazide derivatives **3a–d**, after cyclization with 1 N HCl in benzene furnished the corresponding 4-alkyl-6-phenyl-3(*2H*)-pyridazinone **4a–d** as depicted in Scheme 1.

The structures of the compounds were confirmed by Mass and ^{1}H NMR spectral data. The infrared spectra of compounds (**4a–d**) showed a broad absorption band at 3165–3310 cm^{-1}, which is the characteristic of the NH group and an absorption band at 1650–1660 cm^{-1} for υC=O, characteristic of the amide carbonyl group. The ^{1}H NMR showed a singlet at δ 3.98 ppm region, (for example ^{1}H NMR signal for **4a** observes at $\delta = 3.97$ ppm) which is a characteristic peak for R–CH_2 protons. Various *N*-substituted cyclic amines were reacted with 1-bromo-3-chloropropane and activated zinc under neutral conditions to produce the 1-(3-chloropropyl)-4-substituted cyclic amine derivatives (**A**) (Scheme 2) (Murty *et al.*, 2003).

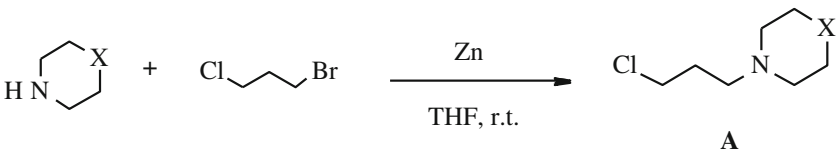
The target compounds 4-substituted-6-phenyl-2-[3-(4-substituted-piperzin-1-yl)-propyl]-pyridazin-3(*2H*)-ones **5a–t** were prepared by treating pyridazinones **4a–d** with the appropriate chloro alkyl substituted cyclic amines with KF-Al_2O_3 in acetonitrile solvent as shown in Scheme 3.

The structures of all the synthesized compounds **5a–t** were confirmed by ^{1}H NMR, IR, and Mass spectral analysis. In the IR Spectrum of the products, the disappearance of a band at 3165–3300 cm^{-1} confirmed that the

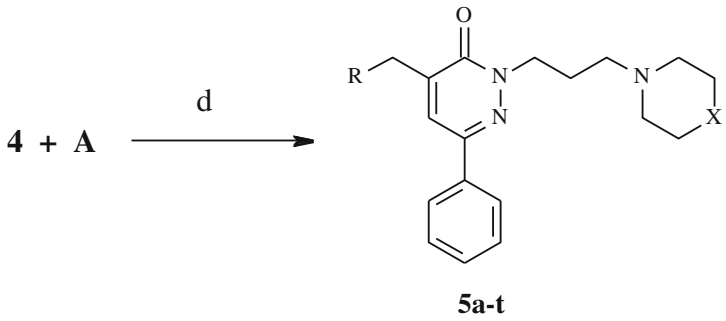


Scheme 1 *R* = phenyl, 2-furyl, 2-pyridyl, 2-thienyl. Reagents and conditions: (a) R–CHO, Ac_2O, NaOMe, 90°C, 2 h; (b) Hydrazine hydrate, MeOH, rt; (c)1.0 N HCl, benzene, rt

Scheme 2 *X* = *N*-phenyl, *N*-benzyl, *N*-ethyl, O, *N*-3-chlorophenyl



Scheme 3 *R* = phenyl, 2-furyl, 2-pyridyl, 2-thienyl; *X* = *N*-phenyl, *N*-benzyl, *N*-ethyl, O, N-3-Chlorophenyl. Reagents and conditions: KF-Al_2O_3, CH_3CN, reflux, 4 h



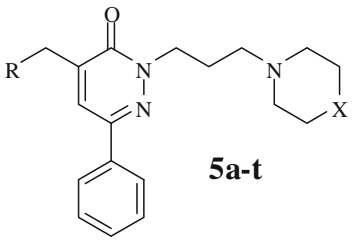
piperazine moiety has substituted at N-2 position of the pyridazinone. The results are summarized in Table 1.

Pyridazinone derivatives **5a–t** can be divided from a structural point of view in three principal parts that may be responsible for pharmacological activity: (i) A pharmacophoric portion constituted by a substituted 6-phenyl-pyridazin-3(*2H*)-one, (ii) A terminal fragment constituted by a cyclic amine moiety and (iii) A three carbon linker between these two substructures.

Cytotoxic activity

The synthesized compounds, **5a–t** were tested for in vitro biological screening for their cytotoxicity toward cancer cell lines using MTT assay as described earlier (Smitha *et al.*, 2005). The cytotoxicity studies were determined against five human cancer cell lines, Cervical (HeLa), Breast (SKBR3), Colon (HCT116), Skin (A375), and Lung (H1299) cell lines and the results were presented in the Tables 2 and 3. These two tables indicate the percentage cytotoxic activity (dose dependent) of the synthesized compounds at concentrations ranging from 50 to 100 μM. Herein, in the Table 4, we represented the IC_{50} values of the compounds **5a–t** against the three cell lines (the cytotoxicity of the compounds against the other two cell lines did not exhibit significant activity). All these compounds possess common 6-phenyl-2H-pyridazin-3(*2H*)-one nucleus. The substitutions at N-2 and C-4 positions of the pyridazinone moiety play an important role in determining the potency of the compounds **5a–t**. Table 4 reveals that

Table 1 Substituted pyridazin-3-(*2H*)-one derivatives (**5a–t**)



5a-t

Compound[a]	*R*	*X*	Yield[b]
5a	Phenyl	–N–C_6H_5	80
5b	2-Furyl	–N–C_6H_5	71
5c	2-Pyridyl	–N–C_6H_5	68
5d	2-Thienyl	–N–C_6H_5	74
5e	Phenyl	–N–CH_2–C_6H_5	82
5f	2-Furyl	–N–CH_2–C_6H_5	75
5g	2-pyridyl	–N–CH_2–C_6H_5	66
5h	2-Thienyl	–N–CH_2–C_6H_5	68
5i	Phenyl	–CH_2CH_3	72
5j	2-Furyl	–CH_2CH_3	70
5k	2-Pyridyl	–CH_2CH_3	70
51	2-Thienyl	–CH_2CH_3	80
5m	Phenyl	–O	74
5n	2-Furyl	–O	77
5o	2-Pyridyl	–O	75
5p	2-Furyl	–O	80
5q	Phenyl	–N–3–Cl–C_6H_4	75
5r	2-Furyl	–N–3–Cl–C_6H_4	78
5s	2-Pyridyl	–N–3–Cl–C_6H_4	72
5t	2-Thienyl	–N–3–Cl–C_6H_4	68

[a] All the compounds were characterized by ^{1}H NMR, IR and mass spectroscopy

[b] Isolated and optimized yields

compounds **5e**, **5i**, **5j**, and **5t** ($IC_{50} < 100$) exhibited good cytotoxicity against the cervical cell line, HeLa, where as the compound **5l** showed maximum cytotoxicity ($IC_{50} = 36$) over the remaining compounds against HeLa cell line in the series. Moderate activity was exhibited by the compounds **5b**, **5d,** and **5e** in SKBR3, **5o** in A375 and **5e** and **5o** in H1299. The compounds **5g** and **5p–t** showed good activity against colon cancer cell line (HCT116). The compounds **5c** and **5j** show moderate activity against HCT116 cell line. By stimulating these findings, we concluded that in addition to the C-4 and N-2 substitution, the type of cell line will also affect the cytotoxicity of the compounds. In case of HCT116 (Colon cancer cell line) substitution at N-2 will be more effective in determining the potency of the compound. This was indicated by IC_{50} values of **5p–t**. Among these compounds **5q**, **5s**, and **5t** have same activity which is comparable with the standard drug. This indicates the negligible effect because of C-4 substitution in the pyridazinone skeleton. Compounds **5i–j** and **5l** exhibited good activity against cervical cancer cell line (HeLa). Compound **5l** ($R = 2$-thienyl and $X = N$-C_2H_5) shows better activity against the same cell line (HeLa). Here, the substitution at C-4 affects the cytotoxicity of the compounds. As far as breast cancer cell line is concerned **5a–e** ($R =$ phenyl or $X = N$-phenyl) showed better cytotoxic activity when compared to other substituents. Hence, the effect because of pipezine moiety is nominal against the breast cancer cell line (SKBR3).

Thus, the activity profile of these pyridazinone-piperzine compounds can be used as new lead molecules in the development of effective anticancer agents.

Conclusions

As part of our continuous search for the potential anticancer heterocyclic compounds, a series of new 4-substituted 6-phenyl pyridazin-3(*2H*)-one derivatives **5a–t** were synthesized and assessed for their anticancer activity. The synthesis involves the Friedel-Crafts acylation of benzene with succinic anhydride followed by various chemical transformations. The *N*-alkylation of the pyridazin-3(*2H*)-ones with chloropropyl piperazines was achieved by using KF/Al_2O_3 in acetonitrile solvent. The products were obtained in high purity with excellent yields. All the newly synthesized pyrdiazin-3(*2H*)-one derivatives were screened for anticancer activity on human cell lines. Hence, our preliminary studies indicate that, the compounds **5q**, **5s**, **5t**, and **5l** are the potent molecules possessing anti-proliferative activity. Compounds **5g**, **5p**, and **5r** also may be considered for further studies in more cell lines. In short, our findings might be beneficial as leads for designing new compounds with potential antitumoral activity. However, further studies are necessary to evaluate the signal transduction pathways induced by these compounds.

Experimental

All the reagents were obtained from commercial sources. Melting points were determined on a Buchi capillary melting point apparatus. The ^{1}H NMR (200 and 300 MHz) and spectra was recorded on Varian Gemini and Bruker Avance spectrometers. Chemical shifts are expressed in ppm down field from internal tetramethyl silane (TMS). The mass spectra were recorded on a VG Auto Spec mass spectrometer. Elemental analyses were performed on Elemental VARIO EL elemental analyzer. IR spectra were recorded on Perkin-Elmer Infrared-683 Infrared spectrometer.

Table 2 The percentage cell viability induced by the compounds **5a–t** in five different cancer cell lines

Compound	Percentage viability of the cells compared to untreated control									
Cell lines	HeLa		SKBR3		HCT116		A375		H1299	
Concentration in μM	50	100	50	100	50	100	50	100	50	100
5a	79.6	77.7	100.0	82.0	100.0	97.0	100.0	100.0	100.0	100.0
5b	75.0	61.3	69.9	45.6	77.8	73.5	98.7	96.5	78.9	78.5
5c	78.2	69.9	72.9	60.5	66.3	56.2	94.2	90.8	88.0	77.6
5d	81.6	77.2	67.1	56.8	78.6	78.5	96.0	93.6	86.2	81.5
5e	67.9	42.7	70.6	59.0	73.4	70.9	91.1	80.1	78.5	71.4
5f	72.5	61.7	80.9	79.9	86.6	86.1	100.0	97.6	95.7	93.0
5g	71.9	63.8	83.6	80.0	54.9	34.3	90.8	85.8	92.4	90.2
5h	80.4	80.0	87.2	75.6	78.4	68.2	98.5	97.0	96.1	95.5
5i	65.1	45.8	88.3	85.1	77.2	75.7	93.0	82.6	96.0	94.7
5j	68.4	42.8	82.8	80.9	72.2	59.5	92.0	90.3	96.4	95.4
5k	70.5	61.9	100.0	92.2	100.0	98.3	93.6	91.0	100.0	97.2
5l	45.2	37.1	96.3	96.2	78.8	66.8	86.8	86.0	98.8	96.0
5m	81.9	61.8	100.0	100.0	94.9	91.3	89.5	89.3	95.2	90.5
5n	70.3	64.6	93.4	90.5	83.6	63.5	90.1	86.3	100.0	90.5
5o	73.1	73.0	96.0	96.2	81.0	78.5	83.8	81.6	82.4	74.3
5p	78.7	67.1	100.0	87.1	49.7	42.3	93.2	93.0	96.1	95.5
5q	72.1	56.6	83.3	75.1	40.3	34.8	83.2	77.1	94.1	92.8
5r	70.1	67.8	80.6	78.5	63.1	42.3	100.0	92.0	94.1	90.1
5s	75.3	72.8	90.2	86.5	43.5	37.0	85.7	81.8	97.3	94.2
5t	67.0	37.8	78.6	72.6	44.0	43.8	84.8	76.5	93.2	92.8

Table 3 The percentage cell viability induced by Curcumin (positive control) in five different cancer cell lines

Cell Line	Concentration in μM		
	5	10	25
HeLa	78.3	53.7	28.0
SKBR3	53.7	74.0	56.5
HCT116	61.6	49.6	22.4
A375	92.0	72.3	50.2
H1299	86.1	62.4	44.7

Chemistry

*General procedure for the synthesis of 3-substituted-5-phenyl-2-yl-methylene-2-(3H)-furanones (**2a–d**)*

A solution of 3-(aroyl) propionic acid (3 mmol) and aromatic aldehyde (equimolar, 3 mmol) in acetic anhydride (15 mL) with anhydrous sodium acetate (4.5 mmol) was refluxed for 4 h under anhydrous conditions. After completion of reaction, the contents were poured into crushed ice in small portions while stirring. A solid mass separated out, which was filtered, washed with water and crystallized from a mixture of methanol/chloroform (1:1) to give (**2a–d**).

Table 4 Cytotoxic activity (IC_{50}, μM) of compounds **5a–t** against three human cancer cell lines

Compound	*R*	*X*	HeLa	SKBR3	HCT116
5a	Phenyl	–N–C_6H_5	150	150	150
5b	2-Furyl	–N–C_6H_5	129	82	150
5c	2-Pyridyl	–N–C_6H_5	150	126	114
5d	2-Thienyl	–N–C_6H_5	150	115	150
5e	Phenyl	–N–CH_2–C_6H_5	78	121	150
5f	2-Furyl	–N–CH_2–C_6H_5	131	150	150
5g	2-Pyridyl	–N–CH_2–C_6H_5	138	150	55
5h	2-Thienyl	–N–CH_2–C_6H_5	150	150	150
5i	Phenyl	–CH_2CH_3	71	150	150
5j	2-Furyl	–CH_2CH_3	79	150	123
5k	2-Pyridyl	–CH_2CH3	131	150	150
5l	2-Thienyl	–CH_2CH_3	36	150	150
5m	Phenyl	–O	131	150	150
5n	2-Furyl	–O	141	150	150
5o	2-Pyridyl	–O	150	150	150
5p	2-Furyl	–O	150	150	49
5q	Phenyl	–N–3–Cl–C_6H_4	115	150	29
5r	2-Furyl	–N–3–Cl–C_6H_4	150	150	67
5s	2-Pyridyl	–N–3–Cl–C_6H_4	150	150	29
5t	2-Thienyl	–N–3–Cl–C_6H_4	76	150	28
Curcumin	_	–	17	27	10

*General procedure for the synthesis of 3-aroyl-2-(substituted-4-yl-methylene)-propionic acid hydrazide (**3a–d**)*

To a solution of the furanones (**2a–d**) (1 mmol) in ethanol (10 mL), hydrazine hydrate (1.3 mmol) was added and maintain the temperature at 10–15°C. The reaction mixture allowed to stand for about 2 h. Evaporate the solvent and pour the reaction mixture into crushed ice. A solid mass is separated. Filter off the solid and washed with water then recrystallized form ethanol.

*General procedure for the synthesis of 4-substituted-6-phenyl-3-(2H)-pyrdidazinone (**4a–d**)*

1.0 N HCl (5 mL) was added drop wise with stirring at 25–35°C over 30–60 min to a solution of the 3-aroyl-2-(substituted-4-yl-methylene)-propionic acid hydrazides (**4a–d**) (3 mmol) in benzene (20 mL), after completion of the addition, the solid was continuously stirred for 1 h, then filtered off and washed thoroughly with water. The product obtained was recrystallized from ethanol.

*4-benzyl-6-phenyl-2H-pyridazin-3-one (**4a**)* Solid. mp.113–115°C; Yield 85%, ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.62 (d, $J = 6.8$ Hz, 2H), 7.39–7.;16 (m, 2H), 6.85 (d, $J = 8.7$ Hz, 2H), 6.79 (t, $J = 7.8$ Hz, 1H), 4.33 (t, $J = 6.8$ Hz, 2H), 3.94 (s, 2H), 3.16–3.07 (m, 4H), 2.62–2.56 (m, 4H), 2.53 (t, $J = 6.8$ Hz, 2H), 2.10 (q, $J = 6.8$ Hz, 2H). IR (KBr): 1649 (C=O), 1602 (C=N) cm^{-1}; ESI–MS: *m/z* 465$(M + H)^+$.

*General procedure for the synthesis of 4-substituted-6-phenyl-2-[3-(4-substituted-piperzin-1-yl)-propyl]-pyridazin-3-(2H)-ones (**5a–t**)*

A mixture of 6-phenyl-3-(2H)-pyridazinone (3.0 mmol) and KF-Al_2O_3 (4.5 mmol) in dry acetonitrile (15 mL) was stirred for 20 min under N_2 atmosphere. 1-(3-chloro-propyl)-4-substituted-piperzin (3.2 mmol) was added to the above mixture and stirred for 5 h. After the completion of reaction (confirmed by TLC), the solvent was evaporated and cold water was added to the reaction mixture and stirred for 30 min. Extract the organic compound with ethyl acetate. The ethyl acetate layer is dried over anhydrous sodium sulfate. The compound was purified by column chromatography on silica gel eluting with EtOAc-*n*-hexane.

*4-Benzyl-6-phenyl-2-[3-(phenyl-piperzin-1-yl)-propyl]-2H-pyridazin-3-one (**5a**)* solid. mp. 113–115°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.62 (d, $J = 6.8$ Hz, 2H), 7.39–7.16, (m, 2H), 6.85 (d, $J = 8.7$ Hz, 2H), 6.79 (t, $J = 7.8$ Hz, 1H), 4.33 (t, $J = 6.8$ Hz, 2H), 3.94 (s, 2H), 3.16–3.07 (m, 4H), 2.62–2.56 (m, 4H), 2.53 (t, $J = 6.8$ Hz, 2H), 2.10 (q, $J = 6.8$ Hz, 2H). IR (KBr): 2945 (C–H), 1649 (C=O), 1602 (C = N) cm^{-1}; ESI–MS: *m/z* 465$(M + H)^+$.

*4-furan-2-ylmethyl-6-phenyl-2-[3-(phenyl-piperzin-1-yl)-propyl]-2H-pyridazin-3-one (**5b**) gummy*

^{1}H NMR ($CDCl_3$, 300 MHz) δ :7.68–7.64 (m, 2H), 7.44–7.19 (m, 10H), 6.34 (m, 1H) 6.22 (d, $J = 3.2$ Hz, 1H), 4.28 (t, $J = 7.2$ Hz, 2H), 3.97 (s, 2H), 2.76–2.50 (m, 8H), 2.34 (t, $J = 8.1$ Hz, 2H), 2.12 (q, $J = 7.2$ Hz, 2H). IR (KBr): 2947 (C–H), 1651 (C=O), 1603 (C=N) cm^{-1}; ESI–MS: *m/z* 455 $(M + H)^+$.

*6-phenyl-2-[3-(phenyl-piperzin-1-yl)-propyl]-4-pyridin-2-ylmethyl-2H-pyridazin-3-one(**5c**) gummy*

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.41 (d, $J = 4.5$ Hz, 1H), 7.69–7.48 (m, 4H), 7.38–7.24 (m, 4H), 7.15–7.02 (m, 3H), 6.76 (d, $J = 8.7$ Hz, 2H), 6.70 (t, $J = 7.2$ Hz, 1H), 4.22 (t, $J = 7.2$ Hz, 2H), 4.02(s, 2H), 3.10–3.00 (m, 4H), 2.55–2.49 (m, 4H), 2.43 (t, $J = 6.8$ Hz, 2H) 2.00 (q, $J = 7.0$ Hz, 2H). IR (KBr): 2927 (C–H), 1649 (C=O), 1598 (C=N) cm^{-1}; ESI–MS: *m/z* 466 $(M + H)^+$.

*6-phenyl-2-[3-(phenyl-piperzin-1-yl)-propyl]-4-thiophen-2-ylmethyl-2H-pyridazin-3-one (**5d**) gummy*

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.72–7.61 (m, 1H), 7.50–7.14, (m, 8H), 6.92 (t, $J = 5.2$ Hz, 1H), 7.02–6.75 (m, 5H), 4.33 (t, $J = 7.2$ Hz, 2H), 4.15 (s, 2H), 3.41 (s, 2H), 3.24–3.10 (m, 4H), 2.70–7.51 (m, 6H), 2.54 (t, $J = 6.6$ Hz, 2H), 2.11 (q, $J = 7.2$ Hz, 2H). IR (KBr): 2923 (C–H), 1649 (C=O), 1600 (C=N) cm^{-1}; ESI–MS: *m/z* 471 $(M + H)^+$.

*4-benzyl-2-[3-(4-benzyl-piperzin-1yl)-propyl]-6-phenyl-2H-pyridazin-3-one (**5e**) gummy*

^{1}H NMR ($CDCl_3$, 300 MHz) δ : 7.67 (d, $J = 7.4$ Hz, 2H), 7.51–7.42 (m, 5H), 7.39–7.16 (m, 6H), 6.85 (d, $J = 8.7$ Hz, 2H), 6.79 (t, $J = 7.8$ Hz, 1H), 4.28 (t, $J = 7.4$ Hz, 2H), 3.93 (s, 2H), 3.47 (s, 2H), 2.54–2.41(m, 8H), 2.35(t, $J = 8.1$ Hz, 2H), 2.04 (q, $J = 8.1$ Hz, 2H IR (KBr): 2937 (C–H), 1650 (C=O), 1604 (C=N) cm^{-1}; ESI–MS: *m/z* 479 $(M + H)^+$.

*2-[3-(4-benzyl-piperzin-1yl)-propyl]-4-furan-2-ylmethyl-6-phenyl-2H-pyridazin-3-one (**5f**) gummy*

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.69–7.64 (m, 2H), 7.43–7.18 (m, 9H), 7.16 (s, 1H), 6.34 (m, 1H), 6.22 (d, $J = 3.2$ Hz, 1H), 4.28 (t, $J = 7.4$ Hz, 2H), 3.97 (s, 2H), 3.50 (s, 2H), 2.54–2.41 (m, 8H), 2.35 (t, $J = 8.1$ Hz, 2H),

2.04 (q, $J = 8.1$ Hz, 2H). IR (KBr): 2924 (C–H), 1650 (C=O), 1602 (C=N) cm^{-1}; ESI–MS: *m/z* 469 (M + 1).

*2-[3-(4-benzyl-piperzin-1yl)-propyl]-6-phenyl-4-pyridn-2-ylmethyl-2H-pyridazin-3-one (**5g**) gummy*

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.51 (d, $J = 4.9$ Hz, 1H), 7.77–7.54 (m, 4H), 7.45–7.09 (m, 10H), 4.26 (t, $J = 7.2$ Hz, 2H), 4.25 (s, 2H), 4.09 (s, 2H), 3.47 (s, 2H), 2.61–2.25 (m, 10H) 2.03 (q, $J = 7.2$ Hz, 2H). IR (KBr): 2939 (C–H), 1650 (C=O), 1601 (C=N) cm^{-1}; ESI–MS: *m/z* 480 $(M + H)^+$.

*2-[3-(4-benzyl-piperzin-1yl)-propyl]-6-phenyl-4-thiophen-2-ylmethyl-2H-pyridazin-3-one (**5h**) gummy*

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.62–7.56 (m, 2H), 7.40–7.12 (m, 10H), 6.92 (t, $J = 5.2$ Hz, 1H), 6.88 (s, 1H), 4.23 (t, $J = 8.3$ Hz, 2H), 4.08 (s, 2H), 3.41 (s, 2H), 2.49–2.28 (m, 10H) 1.99 (q, $J = 6.8$ Hz, 2H). IR (KBr): 2924 (C–H), 1648 (C=O), 1603 (C=N) cm^{-1}; ESI–MS: *m/z* 485$(M + H)^+$.

*4-Benzyl-2-[3-(phenyl-piperzin-1-yl)-propyl]-6-phenyl-2H-pyridazin-3-one (**5i**) gummy*

^{1}H NMR ($CDCl_3$, 500 MHz) δ :7.66–7.60 (m, 2H), 7.45–7.22 (m, 8H), 7.18 (s, 1H), 4.29 (t, $J = 6.8$ Hz, 2H), 3.94 (s, 2H), 2.60–2.42 (m, 10H), 2.40 (q, $J = 6.8$ Hz, 2H), 2.05 (q, $J = 7.8$ Hz, 2H), 1.07 (t, $J = 6.8$ Hz, 3H). IR (KBr): 2960 (C–H), 1647 (C=O), 1602 (C=N) cm^{-1}; ESI–MS: *m/z* 417 $(M + H)^+$.

*2-[3-(4-ethyl-piperzin-1yl)-propyl]-4-furan-2-ylmethyl-6-phenyl-2H-pyridazin-3-one (**5j**) gummy*

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.70–7.65 (m, 2H), 7.45–7.30 (m, 5H), 6.37–6.33 (m, 1H), 6.24–6.21 (m, 1H), 4.29 (t, $J = 7.6$ Hz, 2H), 3.97 (s, 2H), 3.08–3.00 (m, 4H), 2.44–2.70 (m, 12H), 2.05 (q, $J = 6.8$ Hz, 2H), 1.11 (t, $J = 7.6$ Hz, 3H). IR (KBr): 2953 (C–H), 1648 (C=O), 1604 (C=N) cm^{-1}; ESI–MS: *m/z* 407 $(M + H)^+$.

*2-[3-(4-ethyl-piperzin-1yl)-propyl]-6-phenyl-4-pyridin-2-ylmethyl-2H-pyridazin-3-one (**5k**) gummy*

^{1}H NMR ($CDCl_3$, 200 MHz) δ: 7.66 (d, $J = 7.9$ Hz, 2H), 7.51–7.15 (m, 8H), 7.02–6.76 (m, 5H), 4.29 (t, $J = 7.2$ Hz, 2H), 4.15 (s, 2H), 3.25–3.11 (m, 4H), 2.75–2.57 (m, 10H), 2.54 (q, $J = 7.3$ Hz, 2H), 2.05 (q, $J = 7.3$ Hz, 2H), 1.14 (t, $J = 7.2$ Hz, 3H). IR (KBr): 2961 (C–H), 1653 (C=O), 1598 (C=N) cm^{-1}; ESI–MS: *m/z* 429.8 $(M + H)^+$.

*2-[3-(4-ethyl-piperzin-1yl)-propyl]-4-thiophen-2-ylmethyl-6-phenyl-2H-pyridazin-3-one (**5l**) gummy*

^{1}H NMR ($CDCl_3$, 300 MHz) δ :7.83–7.59 (m, 2H), 7.46–7.18 (m, 5H), 6.98 (t, $J = 5.0$ Hz, 1H), 6.34 (m, 1H), 6.94 (s, 1H), 4.29 (t, $J = 7.0$ Hz, 2H), 4.14 (s, 2H), 3.50 (s, 2H), 2.74–2.58 (m, 8H), 2.54 (q, $J = 7.4$ Hz, 2H), 2.06 (q, $J = 8.1$ Hz, 2H), 1.14 (t, $J = 7.2$ Hz, 3H). IR (KBr): 2941 (C–H), 1649 (C=O), 1604 (C=N) cm^{-1}; ESI–MS: *m/z* 413.2 $(M + H)^+$.

*4-Benzyl-2-(3-morpholin-4-ylpropyl)- 6-phenyl-2H-pyridazin-3-one (**5m**) gummy*

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.66–7.46 (m, 2H), 7.41–7.21 (m, 7H), 7.19 (s, 1H), 4.30 (t, $J = 7.4$ Hz, 2H), 3.94 (s, 2H), 3.68–3.58 (m, 4H), 2.52–2.39 (m, 4H) 2.35 (t, $J = 7.0$ Hz, 2H), 2.05 (q, $J = 7.4$ Hz, 2H). IR (KBr): 2925 (C–H), 1649 (C=O), 1603 (C=N) cm^{-1}; ESI–MS: *m/z* 390 $(M + H)^+$.

*4-furan-2-ylmethyl -2-(3-morpholin-4-ylpropyl)-6-phenyl-2H-pyridazin-3-one (**5n**) gummy*

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.70–7.64 (m, 2H), 7.45–7.29 (m, 5H), 6.34(m, 1H) 6.22 (d, $J = 3.2$ Hz, 1H), 4.30 (t, $J = 7.4$ Hz, 2H), 3.97 (s, 2H), 3.67–3.60 (m, 4H), 2.52–2.39 (m, 6H), 2.53 (t, $J = 6.8$ Hz), 2.03 (q, $J = 7.4$ Hz, 2H). IR (KBr): 2926 (C–H), 1655 (C=O), 1606 (C=N) cm^{-1}; ESI–MS: *m/z* 380$(M + H)^+$.

*2-[3-morpholin-4-ylpropyl)-6-phenyl-4-pyridin-2-ylmethyl-2H-pyridazin-3-one (**5o**) gummy*

^{1}H NMR ($CDCl_3$, 300 MHz) δ :8.60 (d, $J = 6.0$ Hz, 1H), 8.10–8.04 (m,1H), 7.96–7.74 (m, 4H), 7.53–7.36 (m, 4H), 4.33 (t, $J = 6.8$ Hz, 2H), 3.75–3.63 (m, 4H), 2.63–2.43 (m, 6H), 2.10 (q, $J = 7.2$ Hz, 2H). IR (KBr): 2925 (C–H), 1654 (C=O), 1596 (C=N) cm^{-1}; ESI–MS: *m/z* 390 $(M + H)^+$.

*2-[3-morpholin-4-ylpropyl)6-phenyl-4-thiophen-2-ylmethyl-2H-pyridazin-3-one (**5p**) gummy*

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.41 (d, $J = 4.5$ Hz, 1H), 7.69–7.48 (m, 4H), 7.38–7.24 (m, 4H), 7.15–7.02 (m, 3H), 6.76 (d, $J = 8.7$ Hz, 2H), 6.70 (t, $J = 7.2$ Hz, 1H), 4.30 (t, $J = 7.0$ Hz, 2H), 4.14 (s, 2H), 3.68–3.59 (m, 4H), 2.50–2.35 (m, 6H), 2.05 (q, $J = 7.2$ Hz, 2H). IR (KBr): 2953 (C–H), 1651 (C=O), 1606 (C=N) cm^{-1}; ESI–MS: *m/z* 396 $(M + H)^+$.

*4-benzyl-2-{3-[4-(3-chlorophenyl)-piperzin-1-yl]-propyl}-6-phenyl-2H-pyridazin-3-one (**5q**) gummy*

^{1}H NMR ($CDCl_3$, 200 MHz) δ: 7.67−7.57 (m, 2H), 7.41–7.20 (m, 6H), 7.18 (s, 1H), 7.10 (t, $J = 8.1$ Hz, 1H), 6.83–6.67 (m, 3H), 4.32 (t, $J = 7.2$ Hz, 2H), 3.93 (s, 2H), 3.19–3.08 (m, 4H), 2.61–2.48 (m, 6H), 2.09 (q, $J = 7.2$ Hz, 2H). IR (KBr): 2925 (C–H), 1649 (C=O), 1598 (C=N) cm^{-1}; ESI–MS: *m/z* 499 $(M + H)^+$.

*2-{3-[4-(3-chlorophenyl)-piperzin-1-yl]-propyl}-4-furan-2ylmethyl-6-phenyl-2H-pyridazin-3-one (**5r**) gummy*

^{1}H NMR ($CDCl_3$, 200 MHz) δ: 7.66–7.59 (m, 2H), 7.42–7.21 (m, 6H), 7.19 (s, 1H), 7.10 (t, $J = 8.1$ Hz, 1H), 6.83–6.66 (m, 3H), 4.33 (t, $J = 7.2$ Hz, 3.95 (s, 2H), 3.20–3.08 (m, 4H), 2.62–2.48 (m, 6H), 2.10 (q, $J = 7.2$ Hz, 2H). IR (KBr): 2922 (C–H), 1650 (C=O), 1597 (C=N), cm^{-1}; ESI–MS: *m/z* 488 $(M + H)^+$.

*2-{3-[4-(3-chlorophenyl)-piperzin-1-yl]-propyl}-6-phenyl-4-pyridin-2ylmethyl-2H-pyridazin-3-one (**5s**) gummy*

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.41 (d, $J = 4.5$ Hz, 1H), 7.69–7.48 (m, 4H), 7.38–7.24 (m, 4H), 7.15–7.02 (m, 3H), 6.76 (d, $J = 8.7$ Hz, 2H), 6.70 (t, $J = 7.2$ Hz, 1H), 4.35 (t, $J = 7.2$ Hz, 2H), 3.97 (s, 2H), 3.28–3.14 (m, 4H), 2.53–2.48 (m, 6H), 2.11 (q, $J = 7.0$ Hz, 2H). IR (KBr): 2925 (C–H), 1654 (C=O), 1594 (C=N) cm^{-1}; ESI–MS: *m/z* 413.2 $(M + H)^+$.

*2-{3-[4-(3-chlorophenyl)-piperzin-1-yl]-propyl}-6-phenyl-4-thiophen-2ylmethyl-2H-pyridazin-3-one (**5t**) gummy*

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.68–7.52 (m, 2H), 7.40–7.12 (m, 9H), 6.88 (t, $J = 4.6$ Hz, 1H), 6.80 (s, 1H), 4.33 (t, $J = 7.4$ Hz, 2H), 4.15 (s, 2H), 3.47 (s, 2H), 3.21–3.06 (m, 4H), 2.62–2.48 (m, 4H), 2.44 (t, $J = 7.4$ Hz, 2H), 2.09 (q, $J = 7.2$ Hz, 2H). IR (KBr): 2924 (C–H), 1651 (C=O), 1597 (C=N) cm^{-1}; ESI–MS: *m/z* 505 $(M + H)^+$.

Evaluation of cytotoxicity of the compounds toward cancer cells

Maintenance of the cells

Among the human cancer cells of various origins, HeLa (Cervical), SKBR3 (Breast), HCT116 (Colon), and A375 (Skin) were obtained from NCCS, Pune and H1299 (Lung) was a gift from Dr. Bharat Aggarwal, MD Anderson cancer Centre, Houston, Texas. All the cells were maintained in DMEM containing 10% FBS with antibiotics and antimycotics at 37°C in a 5% CO_2 atmosphere.

MTT assay

The cytotoxic activity of the compounds was determined by MTT assay as described earlier. This assay measures the percentage viability of the cells in response to different concentrations of the compounds. Active mitochondrial dehydrogenases of living cells convert the water soluble yellow tetrazolium salt to an insoluble purple formazan. The intensity of color developed is an indicator of percentage of viable cells present.

In brief, cells (3000/well) were plated in 96-well plates and kept overnight at 37°C after which, the cells were incubated with and without various concentrations of the compounds (25, 50, 100, and 250 μM). Curcumin was used as the positive control. At the end of the incubation, medium was removed and fresh medium containing 20% MTT solution (2 mg/mL in PBS) was added to each well. After 2 h, 0.1 ml of the extraction buffer (20% SDS and 50% DMF) was added, and the optical density was measured at 570 nm using a plate reader (Bio-Rad) after 1 h and compared with that of the untreated control. The percentage of inhibition of cell viability was determined with reference to the untreated control. The data were subjected to linear regression analysis and the regression lines were plotted for the best straight-line fit. The IC_{50} concentrations were calculated using the respective regression analysis.

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