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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design and synthesis of some thieno[2,3-*c*]pyridazine derivatives of expected anticancer activity

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Abstract Design and synthesis of some new thienopyridazine derivatives as anticancer agents were the goal of this work. Accordingly, a series of novel compounds were synthesized via reacting thienopyridazine carboxylic acid hydrazide with different organic reagents. Twelve novel compounds were selected by National Cancer Institute for a full anticancer screening assay where seven of the investigated compounds showed non-selective broad spectrum and promising activity almost against all cancer cell lines. One of the most active compounds was chosen to be evaluated against 60-cell line panel at five concentration levels and revealed a remarkable growth inhibition activity.

Keywords Antitumour agents · Thieno[2,3-*c*]pyridazines · Cytotoxic activity · Pharmacophores · Improving chemotherapy regimen

Introduction

During the last four decades, intensive research have been conducted on pyridazine chemistry because many derivatives were found to have a wide variety of biological activities, e.g. psychomimetic (Wermuth *et al*., 1989), anti inflammatory (Tewari and Mishra, 2001; Takaya *et al*., 1979), analgesics (Rohet *et al*., 1997), anticonvulsant (Moreau *et al*., 1994), antiasthmatic and bronchodilator (Yamaguchi *et al*., 1995), antihypertensive (Pifferi *et al*., 1975; Tomil *et al*., 1998; McEvoy and Allen, 1974; Curran and Ross, 1974), phosphodiesterase IV inhibitors (Piaz *et al*., 1997; Pieretti *et al*., 2006), antimicrobial (Hassall *et al*., 1979; Radwan and Bakhite, 1999; Kandile *et al*., 2009; Deeb *et al*., 2004; Nagawade *et al*., 2005; Akbas and Beber, 2005; Wu *et al*., 2009), antidiabetic (Mylari *et al*., 2005; Rathish *et al*., 2009), positive inotropic activity(Mertens *et al*., 1990; Combs *et al*., 1992), antianxiety (Lewis *et al*., 2006) and antitumour activity (Malinka and Redzicka, 2004; Byth *et al*., 2004; Brana *et al*., 2005; Shenvei *et al*., 2005). Likewise fused pyridazines, e.g. imidazopyridazines were designed as potent selective CDK2 inhibitors and provided useful leads for discovery and development of CDK inhibitors (Byth *et al*., 2004). Pyrazolopyridazines and their analogues were identified in a high-throughput screening as potent inhibitor of CDK1/cyclin B and have selectivity for the CDK family (Brana *et al*., 2005). Moreover, thieno[2,3-*d*]pyridazines were prepared and found to have I.Kappa.B kinase (IKKs) inhibitors that are useful as anticancer (Shenvei *et al*., 2005).

Thieno[2,3-*c*]pyridazines bearing several pharmacophores that hoping to show anticancer activity were synthesized. Sulphonamide derivatives that have been reported to show substantial antitumour activity in vitro and/or in vivo (Owa *et al*., 1999; Abbate *et al*., 2004), anhydride moiety that showed significant effect as cytotoxic agent for T lymphoblastic leukaemia cell line (Urban *et al*., 2004) and colon tumour cancer (McClusky *et al*., 2000), pyrazolyl, oxadiazolyl and thiadiazolyl moieties that proved to have cytotoxic effect (Khalil *et al*., 2003; Aboraia *et al*., 2006; Loetchutinat *et al*., 2003; Abadi *et al*., 2003; Rostom *et al*., 2003; Abdel-Hamid *et al*., 2007) were used as pharmacophores. Likewise, thiourea and thiosemicarbazones show potent antiproliferative [Khalil *et al*., 2003; Esteves-Souza *et al*., 2006; Dilovic *et al*., 2008) and ribonucleotide reductase inhibitory actions (French *et al*., 1970; Quiroga *et al*., 1998). Moreover, one of the

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promising class of compounds was the Schiff's bases as they are hydrolyzed readily in vitro under mildly acidic conditions, such compounds probably could be hydrolyzed selectively by the tumour cells to liberate the active aldehydes that serve as alkylating agents while the active amine is liberated to act as an antimetabolite (Popp, 1961; Popp and Kirsch, 1961; Modi *et al*., 1970; Billman and Schmidgall, 1970). All of the aforementioned stimulated the interest in synthesizing compounds containing these moieties hoping that these compounds show acceptable value as anticancer agents.

Results and discussion

Chemistry

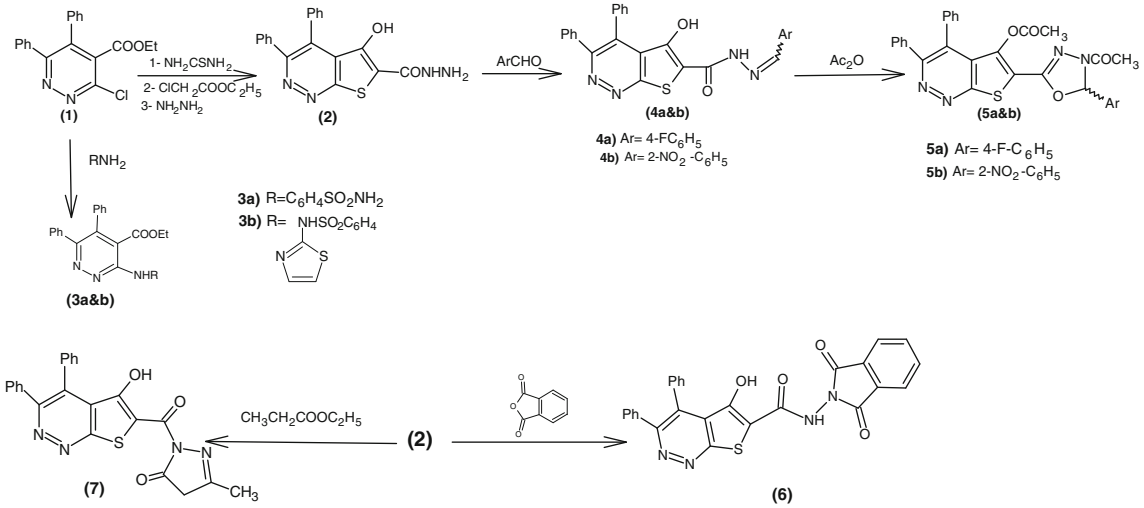
The synthesis of the target compounds is outlined in Schemes 1, 2 and 3. A controlled hydrazinolysis of benzil(1,2-diphenylethane-1,2-dione) gave benzil monohydrazone which upon reacting with diethyl malonate in strongly basic medium followed by chlorination of the resulted product using excess phosphorus oxychloride afforded ethyl 3-chloro-5,6-diphenyl-4-pyridazine-carboxylate **1** (Schmidt and Druey, 1954). In a reported multistep pathway, preparation of compound **2** was carried out by interacting compound **1** with thiourea in ethanol (Kamal El-Dean *et al*., 1996) then the resulted ethyl 2,3-dihydro-5,6-diphenyl-3-thioxo-2,3-dihydro-4-pyridazine-carboxylate was reacted with ethyl chloroacetate to afford the corresponding thieno[2,3-*c*]pyridazine-carboxylate (Radwan and Bakhite, 1999) which upon hydrazinolysis turned into compound **2** (Bakhite *et al*., 2002). Condensation of certain sulphonamide derivatives with compound **1** in 1-butanol gave compounds **3a**, **3b** in good yield. Heating the thieno[2,3-*c*]pyridazine carboxylic acid hydrazide **2** with aromatic aldehydes in boiling absolute ethanol yielded the arylidene derivatives **4a**, **b**. According to the literature, the hydrazones may exist as *Z*/*E* geometrical isomers about C=N and *cis*/*trans* amide conformers; besides hydrazones derived from aldehydes and substituted hydrazides are present in solution in the *E* form. It has been reported that when hydrazones are dissolved in dimethyl sulfoxide (DMSO)-d_6 solution, the *E* geometrical isomers of these compounds undergo a rapid *cis*/*trans* amide equilibrium, in which the *cis* conformer predominates (Gerard *et al*., 1986; Elzbieta and Prukaia, 1998; Salgin-Goksen *et al*., 2007). Boiling **4a**, **b** with excess acetic anhydride for 1 h afforded compounds **5a**, **5b**. Regarding this short reaction time, the *N*-acetyl hydroxy oxadiazolinyl thienopyridazine was expected to be the product, but examining the spectral data showed exclusive formation of the N– and O– diacetyl oxadiazolinyl thienopyridazine **5a**, **b**; IR spectra showed appearance of a strong absorption bands at 1784, 1670 cm^{-1} (2C=O) while the ^1H NMR revealed the absence of singlet signal at δ 8.24 ppm corresponding to the azomethine (CH=N) of its precursor as well as the disappearance of the signals at δ 12.83 and δ 13.07 ppm corresponding to NH and OH, also the presence of two singlet signals at δ 1.42 and δ 1.91 ppm corresponding to the $2COCH_3$ groups. Synthesis of compound **6** was achieved via reacting compound **2** with phthalic anhydride in glacial acetic acid. Reacting ethyl acetoacetate with acid hydrazide **2** in absolute ethanol in the presence of anhydrous sodium acetate afforded compound **7** in a moderate yield.

During the course of this study, oxadiazolylthienopyridazinethiol **8** was prepared via reacting compound **2** with carbon disulphide in basic medium. Alkylation of compound **8** with the appropriate alkyl halide in alcoholic potassium hydroxide yielded exclusively both N– and O–alkylated oxadiazolylthienopyridazine **10a**, **b** and this was confirmed using IR spectra that showed disappearance of (OH) band at 3,365–3,200 cm^{-1} and ^1H NMR spectrum of **10a**, **b** indicated the presence of two singlet signals at δ 2.79 ($S–CH_3$), δ 3.19 ($O–CH_3$) and disappearance of OH signal. It is worth to mention that, upon using anhydrous sodium acetate instead of potassium hydroxide the monoalkylated as well as the dialkylated compounds **9a**, **9b** and **10a**, **10b** were obtained together in fair yield and they are separated by fractional crystallization.

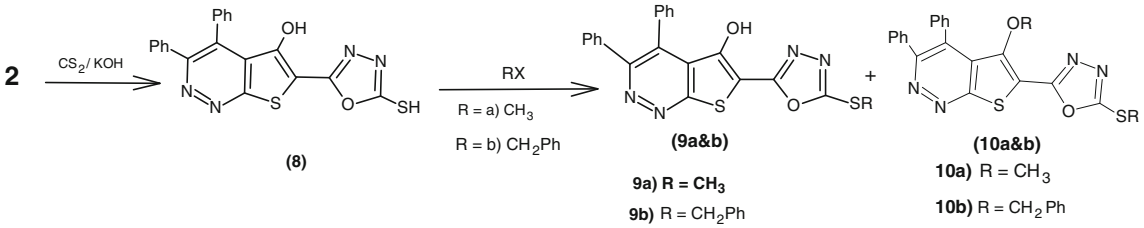
The reaction of acid hydrazide **2** with either methyl isothiocyanate or a mixture of aromatic carboxylic acids and phosphorus oxychloride took place to give compounds **11**, **15a** and **15b**, respectively, in a good yield. Reacting thiosemicarbazide **11** with monochloroacetic acid in glacial acetic acid or heating it with either conc. H_2SO_4 or 2N NaOH afforded the goal compounds **12**, **13** and **14** sequentially. Structure elucidation of the newly synthesized compounds was confirmed using elemental analyses and spectral data.

Preliminary in vitro anticancer screening

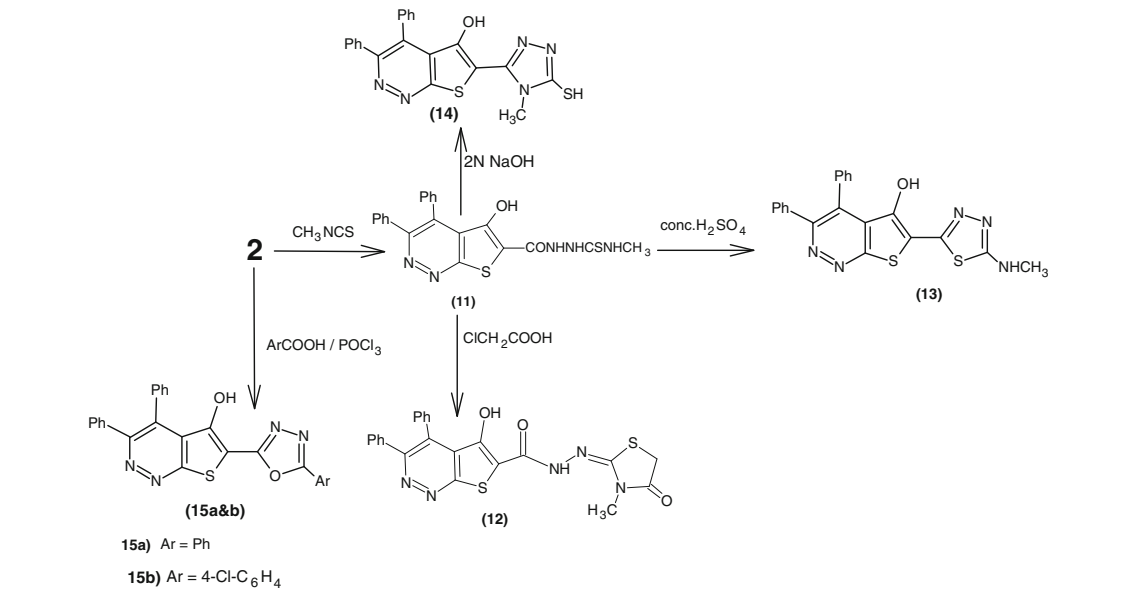
Out of 17 novel compounds, 12 compounds (**3a**, **3b**, **5a**, **5b**, **7**, **8**, **9a**, **9b**, **13**, **14**, **15a** and **15b**) were selected by National Cancer Institute (NCI) to be evaluated. The screening is a two-stage process, beginning with the evaluation of all compounds against the 60-cell lines at a single dose of 10 μM. The operation of this screening utilizes 60 different human tumour cell lines, representing leukaemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate and kidney. The one-dose data was reported as a mean graph of the percent growth of treated cells. Compounds which exhibit significant growth inhibition are evaluated against the 60-cell panel at five concentration



Scheme 1 Synthesis of compounds **3a**&**b**, **4a**&**b**, **5a**&**b**,**6** and **7**



Scheme 2 Synthesis of compounds **8**, **9a**&**b** and **10a**&**b**



Scheme 3 Synthesis of compounds **12**, **13**, **14**, and **15a**&**b**

levels (Monk *et al*., 1991). The test compounds revealed growth inhibition values approximately >40 %, broad spectrum antitumour activity as well as distinctive pattern of selectivity (Table 1). Compound **5b** showed very good activity against leukaemia cell lines; compound **9a** revealed good potency against most cancer cell lines while compound **9b** acted only against leukaemia cell lines. Moreover, compound **7** showed remarkable activity against

Table 1 The mean growth percent, delta value and the growth percent of the synthesized compounds

Cpd. no.	Mean growth percent	Delta	Panel	Sub-panel cell lines (growth %)*
3a	100.57	29.40	Leukaemia	CCRF-CEM (81.76), HL-60 (TB) (71.17), MOLT-4 (78.23), RPMI-8226 (80.92), SR (76.14)
			Non-small cell lung cancer	A549/ATCC (90.89), EKVX (104.79), HOP-62 (108.15), HOP-92 (86.46), NCI-H226 (102.41), NCI-H23 (115.31), NCI-H460 (108.22), NCI-H522 (111.33)
			Colon cancer	COLO 205 (113.16), HCC-2998 (96.78), HCT-116 (93.28), HCT-15 (93.37), HT29 (117.80), KM12 (104.15), SW-620 (104.53)
			CNS cancer	SF-268 (104.65), SF-295 (88.53), SF-539 (107.83), SNB-19 (107.85), SNB-75 (84.61), U251 (95.03)
			Melanoma	LOX IMVI (99.43), M14 (104.44), MDA-MB-435 (107.35), SK-MEL-2 (122.90), SK-MEL-28 (107.03), SK-MEL-5 (94.59), UACC-257 (101.44), UACC-62 (90.20).
			Ovarian Cancer	OVCAR-3 (118.14), OVCAR-5 (116.04), OVCAR-8 (99.79), NCI/ADR-RES (96.78), SK-OV-3 (107.94)
			Renal cancer	ACHN (97.61), CAKI-1 (83.81), RXF 393 (108.65), SN12C (103.81, TK-10 (108.36)
			Prostate cancer	DU-145 (119.58)
			Breast cancer	MDA-MB-231/ATCC (107.99), HS 578T (116.13), BT-549 (94.96), T-47D (91.33), MDA-MB-468 (97.89)
3b	106.03	29.84	Leukaemia	CCRF-CEM (91.26), HL-60 (TB) (104.16), MOLT-4 (79.33), RPMI-8226 (96.51), SR (76.19)
			Non-small cell lung cancer	A549/ATCC (110.47), EKVX (101.97), HOP-62 (116.93), HOP-92 (84.26), NCI-H226 (102.49), NCI-H460 (110.74), NCI-H522 (102.52)
			Colon cancer	COLO 205 (114.19), HCT-116 (98.58), HCT-15 (106.45), HT29 (116.43), KM12 (113.98), SW-620 (131.32)
			CNS cancer	SF-268 (111.63), SF-295 (101.35), SF-539 (116.80), SNB-19 (108.01), SNB-75 (104.30), U251 (98.51)
			Melanoma	M14 (104.03), MDA-MB-435 (119.20), SK-MEL-2 (118.01), SK-MEL-28(113.16), SK-MEL-5 (97.63), UACC-257 (104.13), UACC-62 (92.90)
			Ovarian cancer	OVCAR-3 (119.99), OVCAR-5 (116.92), OVCAR-8 (102.01), SK-OV-3 (105.59)
			Renal cancer	786-0 (109.27), ACHN (102.02), CAKI-1 (106.73), RXF 393 (120.22), SN12C (94.78), TK-10 (109.21)
			Prostate cancer	DU-145 (114.49)
			Breast cancer	MDA-MB-231/ATCC (114.64), HS 578T (117.67), BT-549 (98.88) T-47D (104.38), MDA-MB-468 (110.22)
5a	88.10	48.97	Leukaemia	CCRF-CEM (39.13), HL-60 (TB) (83.79), MOLT-4 (55.07), RPMI-8226 (71.26), SR (79.30)
			Non-small cell lung cancer	EKVX (112.62), HOP-62 (120.56), HOP-92 (46.17), NCI-H226 (92.39), NCI-H23 (102.71), NCI-H322M (85.72), NCI-H460 (101.37), NCI-H522 (80.41)
			Colon cancer	COLO 205 (116.80), HCC-2998 (82.78), HCT-116 (80.50), HCT-15 (61.15) HT29 (101.51), KM12 (81.73), SW-620 (97.41)
			CNS cancer	SF-268 (89.62), SF-539 (104.41), SNB-19 (82.64), SNB-75 (78.06), U251 (104.67)
			Melanoma	LOX IMVI (98.96), M14 (101.54), MDA-MB-435(79.63), SK-MEL-2 (99.88), SK-MEL-28 (82.09), SK-MEL-5 (88.23), UACC-257 (113.18), UACC-62 (57.55)
			Ovarian cancer	OVCAR-3 (96.33), OVCAR-5 (95.35), OVCAR-8 (100.86), NCI/ADR-RES (85.35), SK-OV-3 (97.61)
			Renal cancer	786-0 (94.72), ACHN (71.97), RXF 393 (87.97), SN12C (80.24), TK-10 (94.06), UO-31 (83.34)
			Prostate cancer	DU-145 (114.07)
			Breast cancer	MCF7 (73.65), MDA-MB-231/ATCC (85.91), HS 578T (119.61) BT-549 (65.26), T-47D (89.97), MDA-MB-468 (84.04)
5b	89.12	69.51	Leukaemia	CCRF-CEM (19.61), HL-60 (TB) (58.85), MOLT-4 (47.20), RPMI-8226 (57.43), SR (49.02)
			Non-small cell lung cancer	EKVX (103.17), HOP-62 (108.98), HOP-92 (72.35), NCI-H226 (92.99), NCI-H23 (100.52), NCI-H460 (100.34), NCI-H522 (92.03)
			Colon cancer	COLO 205 (108.41), HCC-2998 (119.54), HCT-116 (76.77), HCT-15 (78.03), HT29 (87.54), KM12 (108.16), SW-620 (82.83)
			CNS cancer	SF-268 (85.56), SF-295(85.05), SF-539 (107.22), SNB-19 (88.60), SNB-75 (76.33), U251 (80.55)
			Melanoma	LOX IMVI (105.36). MALME-3M (93.18), M14 (91.79), MDA-MB-435 (97.87), SK-MEL-2 (101.08), SK-MEL-28 (97.12), SK-MEL-5 (98.48), UACC-257 (100.74), UACC-62 (79.98)
			Ovarian cancer	OVCAR-3 (93.38), OVCAR-5 (95.13), OVCAR-8 (90.12), NCI/ADR-RES (98.39), SK-OV-3 (105.80)
			Renal cancer	786-0 (101.77), ACHN (98.01), CAKI-1 (86.82), RXF 393 (93.45), SN12C (76.26), TK-10 (106.67), UO-31 (78.88)
			Prostate cancer	DU-145 (104.34)
			Breast cancer	MCF7 (64.05), MDA-MB-231/ATCC (89.64), HS 578T (114.02), BT-549 (89.78), T-47D (97.53), MDA-MB-468 (86.49)

Table 1 continued

Cpd. no.	Mean growth percent	Delta	Panel	Sub-panel cell lines (growth %)*
7	81.02	64.98	Leukaemia	CCRF-CEM (49.49), HL-60 (TB) (69.40), MOLT-4 (67.75), RPMI-8226 (59.34), SR (68.57)
			Non-small cell lung cancer	A549/ATCC (84.52), EKVX (74.35), HOP-62 (82.76), HOP-92 (69.54), NCI-H226 (91.86), NCI-H23 (82.61), NCI-H460 (52.21), NCI-H522 (87.44)
			Colon cancer	COLO 205 (111.27), HCC-2998 (59.67), HCT-116 (89.78), HCT-15 (49.03), HT29 (118.61), KM12 (71.30), SW-620 (79.43)
			CNS cancer	SF-268 (88.28), SF-295 (48.77), SF-539 (109.29), SNB-19 (60.71), SNB-75 (90.01), U251 (71.69)
			Melanoma	LOX IMVI (97.61), M14 (87.40), MDA-MB-435 (83.66), SK-MEL-2 (109.63), SK-MEL-28 (83.45), SK-MEL-5 (80.19), UACC-257 (86.80), UACC-62 (77.39)
			Ovarian cancer	OVCAR-3 (84.74), OVCAR-5 (116.72), OVCAR-8 (86.95), NCI/ADR-RES (16.04), SK-OV-3 (90.63)
			Renal cancer	786-0 (65.92), ACHN (73.19), CAKI-1 (31.00), RXF 393 (95.62), SN12C (93.85), TK-10 (103.38)
			Prostate cancer	DU-145 (97.81)
			Breast cancer	MCF7 (85.12), MDA-MB-231/ATCC (121.74), HS 578T (89.56), BT-549 (104.40), T-47D (82.16), MDA-MB-468 (80.49)
8	101.86	29.58	Leukaemia	CCRF-CEM (96.08), HL-60 (TB) (101.19), MOLT-4 (91.02), RPMI-8226 (102.43), SR (83.38)
			Non-small cell lung cancer	A549/ATCC (106.79), EKVX (101.64), HOP-62 (99.71), HOP-92 (72.28), NCI-H226 (91.52), NCI-H460 (116.24), NCI-H522 (101.95)
			Colon cancer	COLO 205 (106.14), HCT-116 (103.46), HCT-15 (103.25), HT29 (113.71), KM12 (118.62), SW-620 (123.96)
			CNS cancer	SF-268 (102.12), SF-295 (111.85), SF-539 (106.47), SNB-19 (103.01), SNB-75 (72.62), U251 (106.06)
			Melanoma	M14 (99.51), MDA-MB-435 (93.59), SK-MEL-2 (106.13), SK-MEL-28 (102.19), SK-MEL-5 (94.73), UACC-257 (97.02), UACC-62 (94.34)
			Ovarian cancer	OVCAR-3 (114.84), OVCAR-5 (101.82), OVCAR-8 (106.44), SK-OV-3 (96.37)
			Renal cancer	786-0 (108.48), ACHN (92.38), CAKI-1 (93.26), RXF 393 (108.60), SN12C (94.04), TK-10 (103.36)
			Prostate cancer	DU-145 (112.35)
			Breast cancer	MCF7 (91.97), MDA-MB-231/ATCC (109.03), HS 578T (121.05), BT-549 (99.45), T-47D (104.90), MDA-MB-468 (107.85)
9a	79.34	38.49	Leukaemia	CCRF-CEM (47.40), HL-60 (TB) (106.92), MOLT-4 (61.85), RPMI-8226 (67.23), SR (62.54)
			Non-small cell lung cancer	A549/ATCC (80.50), EKVX (77.45), HOP-62 (71.09), NCI-H226 (87.66), NCI-H460 (56.66), NCI-H522 (96.63)
			Colon cancer	COLO 205 (110.09), HCT-116 (60.87), HCT-15 (54.62), HT29 (106.98), KM12 (72.87), SW-620 (92.54)
			CNS cancer	SF-268 (80.58), SF-295 (40.85), SF-539 (84.57), SNB-19 (66.39), SNB-75 (78.42), U251 (62.91)
			Melanoma	M14 (73.00), MDA-MB-435 (88.39), SK-MEL-2 (102.24), SK-MEL-28 (85.53), SK-MEL-5 (83.46), UACC-257 (91.64), UACC-62 (58.86)
			Ovarian cancer	OVCAR-3 (86.83), OVCAR-5 (100.68), OVCAR-8 (85.77), SK-OV-3 (85.51)
			Renal cancer	786-0 (88.65), ACHN (83.64), CAKI-1 (43.46), RXF 393 (82.59), SN12C (75.67), TK-10 (100.32)
			Prostate cancer	DU-145 (84.70)
			Breast cancer	MCF7 (83.55), MDA-MB-231/ATCC (90.40), HS 578T (98.44), BT-549 (74.10), T-47D (74.97), MDA-MB-468 (78.93)
9b	100.09	47.62	Leukaemia	CCRF-CEM (66.76), HL-60 (TB) (69.81), MOLT-4 (85.98), RPMI-8226 (92.86), SR (106.06)
			Non-small cell lung cancer	EKVX (122.08), HOP-62 (104.80), HOP-92 (52.47), NCI-H226 (101.27), NCI-H23 (106.21), NCI-H322M (93.59), NCI-H460 (110.68), NCI-H522 (88.49)
			Colon cancer	COLO 205 (121.93), HCC-2998 (96.54), HCT-116 (83.35), HCT-15 (93.63), HT29 (89.80), KM12 (106.95), SW-620 (109.04)
			CNS cancer	SF-268 (102.87), SF-539 (121.97), SNB-19 (103.18), SNB-75 (87.82), U251 (122.20)
			Melanoma	LOX IMVI (83.96), M14 (108.50), MDA-MB-435 (99.43), SK-MEL-2 (93.08), SK-MEL-28 (98.86), SK-MEL-5 (99.71), UACC-257 (134.15), UACC-62 (78.64)
			Ovarian cancer	OVCAR-3 (126.46), OVCAR-5 (98.80), OVCAR-8 (103.87), NCI/ADR-RES (93.49), SK-OV-3 (100.41)
			Renal cancer	786-0 (105.20), ACHN (103.91), RXF 393 (114.20), SN12C (90.04), TK-10 (99.47), UO-31 (92.94)
			Prostate cancer	DU-145 (111.58)
			Breast cancer	MCF7 (82.19), MDA-MB-231/ATCC (97.90), HS 578T (135.35), BT-549 (106.94), T-47D (104.20), MDA-MB-468 (101.16)

Table 1 continued

Cpd. no.	Mean growth percent	Delta	Panel	Sub-panel cell lines (growth %)*
13	104.82	32.80	Leukaemia	CCRF-CEM (109.75), HL-60 (TB) (106.84), MOLT-4 (93.30), RPMI-8226 (112.86), SR (78.25)
			Non-small cell lung cancer	EKVX (112.00), HOP-62 (114.59), HOP-92 (116.37), NCI-H226 (106.31), NCI-H23 (115.18), NCI-H322M (106.45), NCI-H460 (105.54), NCI-H522 (110.52)
			Colon cancer	COLO 205 (131.27), HCC-2998 (98.36), HCT-116 (102.46), HCT-15 (98.86), HT29 (101.73), KM12 (125.99), SW-620 (105.10)
			CNS cancer	SF-268 (105.83), SF-295 (91.70), SF-539 (108.67), SNB-19 (105.21), SNB-75 (87.90)
			Melanoma	LOX IMVI (103.37), MALME-3M (110.67), M14 (107.34), MDA-MB-435 (103.42), SK-MEL-2 (126.39), SK-MEL-28 (111.12), SK-MEL-5 (104.34), UACC-257 (94.51), UACC-62 (97.50)
			Ovarian cancer	OVCAR-3 (116.37), OVCAR-5 (96.45), OVCAR-8 (104.14), NCI/ADR-RES (105.01), SK-OV-3 (97.70)
			Renal cancer	786-0 (108.26), ACHN (107.37), CAKI-1 (72.02), RXF 393 (103.04), SN12C (94.21), TK-10 (108.25)
			Prostate cancer	DU-145 (135.07)
			Breast cancer	MCF7 (88.70), MDA-MB-231/ATCC (104.21), HS 578T (108.53), BT-549 (101.34), T-47D (97.60), MDA-MB-468 (92.62)
14	106.58	20.91	Leukaemia	CCRF-CEM (105.31), HL-60 (TB) (100.69), MOLT-4 (101.26), RPMI-8226 (103.03), SR (107.94)
			Non-small cell lung cancer	A549/ATCC (95.93), EKVX (110.54), HOP-62 (102.20), HOP-92 (101.20), NCI-H226 (98.84), NCI-H23 (106.77), NCI-H460 (105.74), NCI-H522 (102.37)
			Colon cancer	COLO 205 (115.02), HCC-2998 (97.65), HCT-116 (107.29), HCT-15 (108.76), HT29 (108.00), KM12 (112.09), SW-620 (103.31)
			CNS cancer	SF-268 (104.66), SF-295 (88.89), SF-539 (108.81), SNB-19 (123.26),SNB-75 (85.68), U251 (108.41)
			Melanoma	LOX IMVI (104.22), M14 (114.09), MDA-MB-435 (118.87), SK-MEL-2 (106.33), SK-MEL-28 (112.60), SK-MEL-5 (108.06), UACC-257 (95.77), UACC-62 (128.45)
			Ovarian cancer	OVCAR-3 (108.96), OVCAR-5 (106.61), OVCAR-8 (99.73), NCI/ADR-RES (104.19), SK-OV-3 (119.09)
			Renal cancer	786-0 (117.73), ACHN (115.12), CAKI-1 (85.67), RXF 393(114.55), TK-10 (111.81)
			Prostate cancer	DU-145 (117.49)
			Breast cancer	MCF7 (88.50), MDA-MB-231/ATCC (122.22), HS 578T (111.17), BT-549 (98.66), T-47D (106.78), MDA-MB-468 (105.36)
15a	51.52	35.99	Leukaemia	CCRF-CEM (47.72), HL-60 (TB) (83.15), MOLT-4 (47.93), RPMI-8226 (64.77), SR (46.84)
			Non-small cell lung cancer	EKVX (94.12), HOP-62 (73.69), HOP-92 (81.71), NCI-H226 (85.04), NCI-H23 (90.42), NCI-H322M (42.51), NCI-H460 (39.83), NCI-H522 (97.24)
			Colon cancer	COLO 205 (102.17), HCC-2998 (70.39), HCT-116 (47.39), HCT-15 (36.64), HT29 (103.44), KM12 (72.29), SW-620 (64.41)
			CNS cancer	SF-268 (85.51), SF-295 (42.89), SF-539 (68.47), SNB-19 (57.21), SNB-75 (84.39)
			Melanoma	LOX IMVI (65.66), MALME-3M (83.48), M14 (69.63), MDA-MB-435 (78.42), SK-MEL-2 (106.28), SK-MEL-28 (85.15), SK-MEL-5 (71.16), UACC-257 (99.78), UACC-62 (51.06)
			Ovarian cancer	OVCAR-3 (72.38), OVCAR-5 (106.37), OVCAR-8 (66.81), NCI/ADR-RES (18.75), SK-OV-3 (83.00)
			Renal cancer	786-0 (79.92), ACHN (67.04), CAKI-1 (29.12), RXF 393 (85.62), SN12C (61.41), TK-10 (90.60)
			Prostate cancer	DU-145 (106.01)
			Breast cancer	MCF7 (58.02), MDA-MB-231/ATCC (89.19), HS 578T (97.05), BT-549 (74.89), T-47D (81.07), MDA-MB-468 (64.74)
15b	79.27	43.85	Leukaemia	CCRF-CEM (27.56), HL-60 (TB) (52.52), MOLT-4 (35.81), RPMI-8226 (40.41), SR (35.11)
			Non-small cell lung cancer	A549/ATCC (51.18), EKVX (58.45), HOP-62 (51.25), HOP-92 (54.54). NCI-H226 (67.24), NCI-H23 (56.55), NCI-H460 (27.44), NCI-H522 (75.12)
			Colon cancer	COLO 205 (67.44), HCC-2998 (54.66), HCT-116 (19.31), HCT-15 (32.00), HT29 (91.24), KM12 (39.18), SW-620 (35.61)
			CNS cancer	SF-268 (55.21), SF-295 (23.53), SF-539 (55.04), SNB-19 (43.91), SNB-75 (62.75), U251 (32.64)
			Melanoma	LOX IMVI (28.10), M14 (55.28), MDA-MB-435(58.58), SK-MEL-2 (87.07), SK-MEL-28 (71.76), SK-MEL-5 (53.66), UACC-257 (85.02), UACC-62 (37.98)
			Ovarian cancer	OVCAR-3 (25.98), OVCAR-5 (84.85), OVCAR-8 (46.09), NCI/ADR-RES (15.53), SK-OV-3 (61.89)
			Renal cancer	786-0 (61.10), ACHN (53.96), CAKI-1 (24.50), RXF 393 (72.35), TK-10 (59.00)
			Prostate cancer	DU-145 (71.93)
			Breast cancer	MCF7 (33.46), MDA-MB-231/ATCC (77.91), HS 578T (62.42), BT-549 (56.05), T-47D (52.95), MDA-MB-468 (44.61)

[a] Growth inhibition (GI %) = 100 % − growth %

Table 2 GI_{50}, TGI, LC_{50} of compound **15b**

Panel/cell line	GI_{50}	TGI	LC_{50}
Leukaemia			
CCRF-CEM	0.00288	0.018316	0.018316
HL-60 (TB)	0.004608	0.018316	0.018316
K-562	0.004169	0.018316	0.018316
MOLT	0.004472	0.018316	0.018316
RPMI	0.003966	0.018316	0.018316
SR	0.004517	0.018316	0.018316
Non-small cell lung cancer			
A549/ATCC	0.004654	0.014846	0.018316
EKVX	0.004046	0.011221	0.018316
HOP-62	0.004992	0.018316	0.018316
HOP-92	0.003215	0.006738	0.018316
NCI-H226	0.003058	0.018316	0.018316
NCI-H23	0.004942	0.018316	0.018316
NCI-H322M	0.004169	0.010567	0.018316
NCI-H460	0.003151	0.008067	0.018316
NCI-H522	0.004339	0.009755	0.018316
Colon cancer			
COLO 205	0.004701	0.018316	0.018316
HCC-2998	0.004992	0.018316	0.018316
HCT-116	0.003966	0.007155	0.014552
HCT-15	0.002265	0.018316	0.018316
HT29	0.004562	0.009095	0.018316
KM12	0.004701	0.014996	0.018316
SW-620	0.004562	0.006605	0.018316
CNS cancer			
SF-268	0.005517	0.018316	0.018316
SF-295	0.00328	0.008148	0.018316
SF-539	0.004748	0.015764	0.018316
SNB-19	0.004701	0.018316	0.018316
SNB-75	0.005742	0.018316	0.018316
U251	0.003553	0.018316	0.018316
Melanoma			
LOX IMVI	0.003735	0.009466	0.018316
MALME-3M	0.004608	0.011333	0.018316
M14	0.004992	0.012155	0.018316
MDA-MB-435	0.004654	0.018316	0.018316
SK-MEL-2	0.004128	0.00823	0.017597
SK-MEL-28	0.001136	0.018316	0.018316
SK-MEL-5	0.005407	0.009095	0.013569
UACC-257	0.006158	0.014996	0.018316
UACC-62	0.003773	0.006605	0.018316
Ovarian cancer			
IGROV1	0.003661	0.006346	0.018316
OVCAR-3	0.003773	0.006097	0.018316
OVCAR-4	0.004748	0.018316	0.018316
OVCAR-5	0.002177	0.018316	0.018316
OVCAR-8	0.004383	0.018316	0.018316
NCI/ADR-RES	0.00258	0.018316	0.018316
SK-OV-3	0.005042	0.018316	0.018316
Renal cancer			
786-0	0.005858	0.018316	0.018316
A498	0.005917	0.012525	0.018316
ACHN	0.004254	0.017422	0.018316
CAKI-1	0.001836	0.009952	0.018316
RXF 393	0.003661	0.018316	0.018316
SN12C	0.004654	0.017597	0.018316
TK-10	0.005144	0.016907	0.018316
UO-31	0.003625	0.00622	0.018316
Prostate cancer			
PC-3	0.004087	0.018316	0.018316
DU-145	0.00622	0.018316	0.018316
Breast cancer			
MCF7	0.004427	0.018316	0.018316
MDA-MB-231/ATCC	0.004562	0.012277	0.018316
HS 578T	0.004427	0.018316	0.018316
BT-549	0.001008	0.018316	0.018316
T-47D	0.004893	0.018316	0.018316
MDA-MB-468	0.005042	0.010462	0.018316

colon cancer HCC-2998, HCT-15 cell lines, CNS cancer SF-295 cell lines and renal cancer CAKI-1 cell lines but selectively acted against ovarian cancer NCI/ADR-RES cell line in an excellent potency. It has to be mentioned that compounds **15a** and **15b** are the most potent ones of the target compounds as they showed activity against almost all cancer cell lines (Table 1). Compound 5-hydroxy-3,4-diphenyl-6-(5-(4-chloro phenyl)-[1,3,4]oxadiazol-2-yl)-thieno[2,3-*c*]pyridazine (**15b**) satisfied a pre-determined threshold inhibition criteria in a minimum number of cell lines, so it was passed to the full five-dose assay on to further evaluation at five concentration levels (0.01 and 100 μM) (Table 2). It showed broad spectrum anticancer activity against all tested sub-panel tumour cell lines, with GI_{50} full panel mean graph mid-point (MG-MID) = −5.4 μM, total growth inhibition (TGI) full panel (MG-MID) = −4.28 μM and LC_{50} full panel (MG-MID) = −4.01 μM.

Experimental

General

Reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel sheets that precoated with

UV fluorescent silica (MERCK 60 F 254) and spots were developed using I_2 vapour/UV light as visualizing agents. Solvent system was chloroform:methanol (in different ratios). ^{1}H NMR spectra were determined in $CDCl_3$, or DMSO-d_6 solvent with Varian Gemini 300 MHZ Spectrometer. Peak positions were given in parts per million (δ) downfield the tetramethylsilane (TMS) as internal standard. IR spectra were recorded on a Shimadzu 435 Spectrometer, using KBr discs and values were represented in cm^{-1}. Mass spectra were run on Hewlett Packard 5988 spectrometer at the Microanalytical Center, Cairo University. Melting points were determined on a Griffin instrument and are uncorrected. All reported products showed ^{1}H NMR spectra in agreement with the assigned structures. Elemental analyses were performed at the Micro-analytical Center, Cairo University, Egypt. Compounds **1** and **2** were prepared adopting a reported procedure (Radwan and Bakhite, 1999; Kamal El-Dean *et al*., 1996; Bakhite *et al*., 2002).

Chemistry

*General procedure for the preparation of compounds **3a** and **3b***

A mixture of **1** (1.69 g, 0.005 mol) and the appropriate 4-aminobenzenesulphonamide (0.005 mol) in 1-butanol (20 mL) was refluxed for 3 h, the reaction mixture was cooled, filtered and the separated solid was crystallized from ethanol.

*Ethyl 5,6-diphenyl-3-(4-sulfamoylphenylamino)-4-pyridazine-carboxylate **3a*** Yield 85 %. mp 230–232 °C. IR (KBr): 3400, 3373, 3300 (NH_2, NH), 3090–3040, (CH, arom), 2910–2850 (CH, aliph), 1747 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, δ, ppm): 0.82–0.88(t, $J = 7.2$ Hz, 3H, –CH_2–CH_3); 4.01–4.12 (q, $J = 7.2$ Hz, 2H, CH_2–CH_3); 7.17–7.94 (m, 15H, Ar–H^+ (1H, NH, D_2O exchangeable); 9.03 (s, 2H, NH_2, D_2O exchangeable) ppm; EIMS: *m/z* (%) = 474(M^+, 35), 473(M^+-1, 100); Anal. Calcd for $C_{25}H_{22}N_4O_4S$: C 63.28, H 4.67, N 11.80; Found: C 63.33, H 4.45, N 11.99 %.

*Ethyl 5,6-diphenyl-3-[4-(thiazol-2-ylsulfamoyl)-phenylamino]-4-pyridazine-carboxylate **3b*** Yield 79 %. mp 191–193 °C. IR (KBr): 3400 (NH), 3066–3026 (CH, arom), 2980–2900 (CH, aliph), 1732 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, δ, ppm): 0.71–0.79 (t, $J = 7.2$ Hz, 3H, –CH_2–CH_3); 4.01–4.10 (q, $J = 7.2$ Hz, 2H, –CH_2–CH_3); 6.82 (s, 2H, C-4, C-5 thiazole), 7.14–7.82 (m, 14H, Ar–H); 9.09 (s, 1H, NH, D_2O exchangeable); 12.72 (s, H, NH, D_2O exchangeable) ppm; EIMS: *m/z* (%) = 558 ($M+1^+$, 2.40); Anal. Calcd for $C_{28}H_{23}N_5O_4S_2$: C 60.31, H 4.16, N 12.56; Found: C 60.30, H 4.00, N 12.64 %.

*General procedure for the preparation of compounds **4a** and **4b***

A mixture of **2** (3.62 g, 0.01 mol) and the appropriate aromatic aldehyde (0.01 mol) in absolute ethanol (10 mL) was heated under reflux for 3 h then cooled, filtered and the solid obtained was crystallized from acetic acid.

*5-Hydroxy-3,4-diphenyl-thieno[2,3-c]pyridazine-6-carboxylic acid (ZE) (4-fluorobenzylidene)-hydrazide **4a*** Yield 80 %; mp 290–292 °C. IR (KBr): 3150 (NH), 3047–3022 (CH, arom), 1631 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, δ, ppm):7.15–7.97 (m, 14H, Ar–H), 8.24 (s, 1H, N=CH), 12.54 (s, 1H, NH, D_2O exchangeable), 13.22 (s, 1H, OH, D_2O exchangeable) ppm; EIMS: *m/z* (%) = 468 (M^+, 6.4), 467 ($M-1^{+\cdot}$, 36.0); Anal. Calcd for $C_{26}H_{17}FN_4O_2S$: C 66.65, H 3.66, N 11.96; Found: C 66.50, H 3.72, N 12.20 %.

*5-Hydroxy-3,4-diphenylthieno[2,3-c]pyridazine-6-carboxylic acid (ZE) (2-nitrobenzylidene)-hydrazide **4b*** Yield 65 %. mp 275–277 °C. IR (KBr): 3100 (NH), 3051–3020 (CH, arom), 1639 (C=O), 1528, 1340 (NO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6, δ, ppm): 7.29–8.32 (m, 14H, Ar–H), 8.67 (s, 1H, N=CH), 12.83 (s, 1H, NH, D_2O exchangeable), 13.07 (s, 1H, OH, D_2O exchangeable) ppm; EIMS: *m/z* (%) = 496($M+1^+$, 6.1), 495 (M^+, 22.2), 331(100 %); Anal. Calcd for $C_{26}H_{17}N_5O_4S$: C 63.02, H 3.46, N 14.13; Found: C 63.09, H 3.81, N 14.12 %.

*General method for preparation of compounds **5a** and **5b***

A mixture of **4a** and **4b** (0.01 mol) and acetic anhydride (3 mL) was refluxed for 1 h, allowed to cool, and then ammonium hydroxide (5 mL) was added. The obtained solid was filtered, washed with water and crystallized from aqueous ethanol (50 %).

*Acetic acid 6-[4-acetyl-5-(4-fluorophenyl)-4,5-dihydro-[1,3,4]oxadiazol-2-yl]-3,4-diphenylthieno[2,3-c]pyridazin-5-yl ester **5a*** Yield 72 %. mp 155–157 °C. IR (KBr): 3057–3032 (CH, arom), 2922–2850 (CH, aliph), 1782, 1676 (2C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6, δ, ppm): 1.42 (s, 3H, –CO CH_3), 1.91 (s, 3H, –CO CH_3), 6.93 (s, 1H, C-2 oxadiazoline), 7.00–8.10 (m, 14H, ArH) ppm; Anal. Calcd for $C_{30}H_{21}FN_4O_4S$: C 65.21, H 3.83, N 10.14; Found: C 64.90, H 3.61, N 10.01 %.

*Acetic acid 6-[4-acetyl-5-(2-nitrophenyl)-4,5-dihydro-[1,3,4]oxadiazol-2-yl]-3,4-diphenylthieno[2,3-c]pyridazin-5-yl ester **5b*** Yield 71 %. mp 228–230 °C. IR (KBr): 3057–3028 (CH, arom), 2910–2820 (CH, aliph) 1784, 1678 (2C=O),

1531, 1344 (NO_2) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 1.32(s, 3H, –CO CH_3), 1.89 (s, 3H, –$COCH_3$), 6.83(s, 1H, C-2 oxadiazoline) 6.87–8.30 (m, 14H, ArH) ppm; EIMS: $m/z(\%) = 580$ ($M+1^+$, 2.92), 579 (M^+, 6.41), 536 (M^+–$COCH_3$, 2.12); Anal. Calcd for $C_{30}H_{21}N_5O_6S$: C 62.17, H 3.65, N 12.08; Found: C 62.60, H 3.80, N 11.88 %.

N-[5-Hydroxy-3,4-diphenylthieno[2,3-c]pyridazine-6-carboxylic acid amido]isoindole-1,3-dione ***6***

To a mixture of **2** (3.62 g, 0.01 mol) in glacial acetic acid (10 mL), phthalic anhydride (2.22 g, 0.015 mol) was added and the mixture was heated under reflux for 6 h, after cooling the reaction mixture was poured onto crushed ice while stirring. The formed precipitate was filtered and crystallized from ethanol to give compound **6** in 3.33 g (68 %) yield. mp 215–217 °C. IR (KBr): 3305, 3200 (OH, NH), 3082–3050 (CH, arom), 1743 (C=O), 1685 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 7.21–8.22 (m, 14 Ar–H), 10.28 (s, 1H, NH, D_2O exchangeable), 11.51(s, 1H, OH, D_2O exchangeable) ppm; EIMS: m/z (%) = 493($M+1^+$, 1.33), 492 (M^+, 4.24 %); Anal. Calcd for $C_{27}H_{16}N_4O_4S$: C 65.84, H 3.27, N 11.38; Found: C 65.84, H 3.25, N 11.30 %.

5-Methyl-2-(5-hydroxy-3,4-diphenylthieno[2,3-c]pyridazine-6-carbonyl)-2,4-dihydro-pyrazol-3-one ***7***

A mixture of **2** (3.62 g, 0.01 mol), anhydrous sodium acetate (0.82 g, 0.01 mol) and ethyl acetoacetate (2.6 g, 0.02 mol) in absolute ethanol (20 mL) was heated under reflux for 8 h, then allowed to cool and poured into ice-cold water (50 mL). The solid product was collected by filtration, dried and crystallized from ethanol to give compound **7** in 0.9 g (76 %) yield. mp 180–182 °C. IR (KBr): 3200 (OH), 3055–3035 (CH, arom), 2993–2930 (CH aliph), 1690 and 1660 (2C=O) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 1.32 (s, 3H, CH_3),4.15–4.40 (dd, $J = 8.3$ Hz, $J = 8.3$ Hz, 2H, C-4 pyrazolone), 7.11–7.31(m, 10H, Ar–H), 10.57 (s, 1H, OH, D_2O exchangeable) ppm; EIMS: m/z (%) = 429($M+1^+$, 2.3), 428(M^+, 2.5 %); Anal. Calcd for $C_{23}H_{16}N_4O_3S$: C 64.47, H 3.76, N 13.08; Found: C 64.70, H 3.94, N 12.80 %.

6-(5-Thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-5-hydroxy-3,4-diphenylthieno[2,3-c]pyridazine ***8***

To a mixture of **2** (3.62 g, 0.01 mol) and potassium hydroxide (0.56 g, 0.01 mol) in aqueous ethanol (50 %, 30 mL), carbon disulphide (12.5 mL) was added and the reaction mixture was refluxed for 8 h, then evaporated under reduced pressure. The residue was dissolved in cold water (30 mL), and dilute HCl (10 mL) was added. The separated solid was filtered, dried and crystallized from acetone to give **8** in 2.7 g (66 %) yield. mp 308–310 °C. IR (KBr): 3321 (OH), 3055–3022 (CH, arom), 2534 (SH) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 7.1–7.3 (m, 10H, Ar–H), 8.4 (s, 2H, OH and SH, D_2O exchangeable) ppm; EIMS: m/z (%) = 406 ($M+2^+$, 4.43), 405 ($M+1^+$, 8.64), 404 (M^+, 25.14); Anal. Calcd for $C_{20}H_{12}N_4O_2S_2$: C 59.39, H 2.99, N 13.85; Found: C 59.19, H 3.08, N 13.62 %.

General method for preparation of compounds ***9a, 9b*** *and* ***10a, 10b***

A mixture of **8** (4.04 g, 0.01 mol), the corresponding alkyl halide (0.01 mol) and anhydrous sodium acetate (0.82 g, 0.01 mol) in absolute ethanol (30 mL) was refluxed for 1 h; the reaction mixture was allowed to cool, and poured into ice-cold water (50 mL). The solid product was collected and fractionally crystallized from ethanol; the insoluble compounds were **9a** and **9b** while the soluble compounds were **10a** and **10b**.

6-(5-Methylsulfanyl-[1,3,4]oxadiazol-2-yl)-3,4-diphenylthieno[2,3-c]pyridazin-5-ol ***9a*** Yield 30 %. mp 240–242 °C. IR (KBr): 3267 (OH), 3057–3028 (CH arom), 2950–2850 (CH aliph) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 2.90 (s, 3H, –S–CH_3), 7.10–7.30 (m, 10H, Ar–H), 7.96 (s, 1H, OH, D_2O exchangeable) ppm; EIMS: m/z (%) = 419($M+1^+$, 16.9), 418(M^+, 45.8); Anal. Calcd for $C_{21}H_{14}N_4O_2S_2$: C 60.27, H 3.37, N 13.39; Found: C 60.08, H 3.57, N 13.50 %.

6-(5-Benzylsulfanyl-[1,3,4]oxadiazol-2-yl)-3,4-diphenylthieno[2,3-c]pyridazin-5-ol ***9b*** Yield 35 %. mp 190–192 °C. IR (KBr): 3300 (OH), 3059–3028 (CH, arom) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 4.11 (s, 2H, CH_2–Ph), 6.75–7.30 (m, 15H, Ar–H), 8.90 (s, 1H, OH, D_2O exchangeable) ppm; Anal. Calcd for $C_{27}H_{18}N_4O_2S_2$: C 65.57, H 3.67, N 11.33; Found: C 65.40, H 3.50, N 11.44 %.

5-Methoxy-6-(5-methylsulfanyl-[1,3,4]oxadiazol-2-yl)-3,4-diphenylthieno[2,3-c]pyridazine ***10a*** Yield 60 %. mp 200–202 °C. IR (KBr): 3066–3055 (CH, arom), 2972–2872 (CH, aliph) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 2.79(s, 3H, –S–CH_3), 3.19 (s, 3H, –O–CH_3), 7.2–7.3(m, 10 H, Ar–H) ppm; Anal. Calcd for $C_{22}H_{16}N_4O_2S_2$: C 61.06, H 3.73, N 12.95; Found: C 61.34, H 3.88, N 12.67 %.

5-Benzyloxy-6-(5-benzylsulfanyl-[1,3,4]oxadiazol-2-yl)-3,4-diphenylthieno[2,3-c]pyridazine ***10b*** Yield 55 %. mp 185–187 °C. IR (KBr): 3047–3035 (CH, arom), 2950–2840

(CH, aliph) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 4.31 (s, 2H–CH_2–Ph), 4.49 (s, 2H, O–CH_2–Ph), 6.90–7.30 (m, 20H, Ar–H) ppm; Anal. Calcd for $C_{34}H_{24}N_4O_2S_2$: C 69.84, H 4.14, N 9.58; Found: C 69.41, H 4.29, N 9.03 %.

1-(5-Hydroxy-3,4-diphenyl-thieno[2,3-c]pyridazin-6-yl)-4-methylthiosemicarbazide ***11***

A mixture of the acid hydrazide **2** (3.62 g, 0.01 mol) and methyl isothiocyanate (0.73 g, 0.01 mol) in dioxin (30 mL) was refluxed for 6 h, the solvent was removed under vacuum. The residue was washed with water, and crystallized from acetone to give compound **11** in 3.4 g (81 %) yield. mp 192–194 °C. IR (KBr): 3329, 3251 (OH, NH), 3053–3030 (CH, arom), 2972–2870 (CH, aliph), 1633 (C=O), 1202 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 2.1 (s, 3H, CH_3), 7.1–7.3 (m, 10H, Ar–H), 11.48, 12.20, 13.05 (3s, 3H, NH, D_2O exchangeable),13.41 (s, 1H, OH, D_2O exchangeable) ppm; Anal. Calcd for $C_{21}H_{17}N_5O_2S_2$: C 57.91, H 3.93, N 16.08; Found: C 57.99, H 4.20, N 16.35 %.

5-Hydroxy-3,4-diphenylthieno[2,3-c]pyridazine-6-carboxylic acid (ZE) (3-methyl)-4-oxothiazolidin-2-ylidene) hydrazide ***12***

A mixture of the thiosemicarbazide **11** (4.35 g, 0.01 mol), monochloroacetic acid (0.94 g, 0.01 mol) and anhydrous sodium acetate (0.82 g, 0.01 mol) in acetic acid (20 mL) was heated under reflux for 10 h, the reaction mixture was cooled and poured into ice-cold water. The precipitate was filtered, washed with water and crystallized from ethanol to give compound **12** in 3.08 (65 %) yield. mp 212–214 °C. IR (KBr): 3300–3200 (OH, NH), 3055–3026 (CH, arom), 2922–2850 (CH, aliph), 1728 (C=O), 1620 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 2.23 (s, 3H, –CH_3), 4.19 (s, 2H, C-5 thiazolidinone),7.11–7.38 (m, 10H, Ar–H), 11.67 (s, 1H, NH, D_2O exchangeable), 13.48(s, 1H, OH, D_2O exchangeable) ppm; EIMS: *m/z* (%) = 476 ($M+1^+$, 11.09), 475 $(M)^+$, 31.47; Anal. Calcd for $C_{23}H_{17}N_5O_3S_2$: C 58.09, H 3.60, N 14.73; Found: C 58.28, H 3.80, N 14.90 %.

5-Hydroxy-6-(5-methylamino-1,3,4-thiadiazol-2-yl)-3,4-diphenylthieno[2,3-c]pyridazine ***13***

An ice-cold solution of the thiosemicarbazide **11** (4.35 g, 0.01 mol) in concentrated sulphuric acid (10 mL) was stirred for 10 min, then left at room temperature for another 10 min. The resulting solution was poured slowly into ice-cold water and made alkaline with conc. ammonia (30 %).The precipitated product was filtered, washed with water and crystallized from ethanol to give compound **13** in 2.89 g (69 %) yield. mp 298–300 °C. IR (KBr): 3203, 3113 (OH, NH), 3050–3029 (CH, arom), 2940–2860 (CH, aliph) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 2.19 (s, 3H, CH_3),7.15–7.35 (m, 10H, Ar–H), 11.55 (s, H, NH, D_2O exchangeable), 13.47 (s, 1H, OH, D_2O exchangeable) ppm; EIMS: *m/z* (%) = 417(M^+, 100); Anal. Calcd for $C_{21}H_{15}N_5OS_2$: C 60.41, H 3.62, N 16.77; Found: C 60.10, H 3.80, N 16.71 %.

6-(4-Methyl-5-thio-4H-1,2,4-triazol-5-yl)5-hydroxy-3,4-diphenylthieno[2,3-c]pyridazine ***14***

A mixture of the thiosemicarbazide **11** (4.35 g, 0.01 mol) and NaOH (2N, 5 mL) was refluxed for 3 h, after cooling the reaction mixture was acidified with dilute HCl. The solid formed was filtered and crystallized from ethanol to give compound **14** in 3.33 g (80 %) yield. mp 142–144 °C. IR (KBr): 3300–3200 (OH, NH), 3056–3024 (CH, arom), 2920–2855 (CH, aliph) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 2.96 (s, 3H, CH_3),7.1–7.2 (m, 10H, Ar–H), 8.00 (s, 2H, OH or SH, D_2O exchangeable) ppm; Anal. Calcd for $C_{21}H_{15}N_5OS_2$: C 60.41, H 3.62, N 16.77; Found: C 60.27, H 3.89, N 16.42 %.

General method for preparation of compounds ***15a*** *and* ***15b***

A mixture of acid hydrazide **2** (3.62 g, 0.02 mol) and the respective aromatic acid (0.02 mol) in phosphorus oxychloride (10 mL) was heated under reflux for 4 h. After cooling, the reaction mixture was poured onto crushed ice and sodium carbonate solution (20 %) (10 mL) was added. The formed precipitate was filtered, washed with water, dried and crystallized from DMF to give **15a** and **15b**.

5-Hydroxy-3,4-diphenyl-6-(5-phenyl-1,3,4-oxadiazol-2-yl)-thieno[2,3-c]pyridazine ***15a*** Yield 80 %.mp 122–124 °C. IR (KBr): 3200 (OH), 3057–3020 (CH, arom) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 6.90–7.33 (m, 15H, Ar–H), 13.35 (s, 1H, OH, D_2O exchangeable) ppm; EIMS: *m/z* (%) = 449(M^++1, 28.35), 448 (M^+, 87.86); Anal. Calcd for $C_{26}H_{16}N_4O_2S$: C 69.63, H 3.60, N 12.49; Found: C 69.60, H 3.88, N 12.16 %.

5-Hydroxy-3,4-diphenyl-6-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-thieno[2,3-c]pyridazine ***15b*** Yield 69 %. mp 192–194 °C. IR (KBr): 3330 (OH), 3093–3053 (CH, arom) cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 7.11–7.93 (m, 14H, Ar–H), 13.20 (s, 1H, OH, D_2O exchangeable) ppm; Anal. Calcd for $C_{26}H_{15}ClN_4O_2S$: C 64.66, H 3.13, N 11.60; Found: C 64.32, H 3.39, N 11.74 %.

Antitumour screening

Under a sterile condition, cell lines were grown in RPMI 1640 media supplemented with 10 % foetal bovine serum, up to 105 cells/mL was used to test the growth inhibition activity of the synthesized compounds. The concentrations of the compounds ranging from 0.01 to 100 μM were prepared in phosphate buffer saline. Each compound was initially solubilized in DMSO; however, each final dilution contained less than 1 % DMSO. Solutions of different concentrations (0.2 mL) were pipetted into separate well of a microtitre tray in duplicate. Cell culture (1.8 mL) containing a cell population up to 104 cells/mL was pipetted into each well. Controls, containing only phosphate buffer saline and DMSO at identical dilutions, were also prepared in the same manner. These cultures were incubated in a humidified incubator at 37 °C. The incubator was supplied with 5 % CO_2 atmosphere. After 48 h, cells in each well were diluted 10 times with saline and counted by using a coulter counter. The counts were corrected for the dilution (Monk *et al*., 1991). The anticancer activity of tested compounds is given by three parameters for each cell line: log GI_{50} value (GI_{50} = molar concentration of the compound that inhibits 50 % net cell growth), log TGI value (TGI = molar concentration of the compound leading to total inhibition) and log LC_{50} value (LC_{50} = molar concentration of the compound leading to 50 % net cell death). Using the seven absorbance measurements [time zero (T_z), control growth (C) and test growth in the presence of drug at the five concentration levels (T_i)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

$[(T_i - T_z)/(C - T_z)]$
$\times 100$ for concentrations for which $T_i \geq T_z$

$[(T_i - T_z)/T_z] \times 100$ for concentrations for which $T_i < T_z$.

Three-dose response parameters are calculated for each experimental agent. Growth inhibition of 50 % (GI_{50}) is calculated from $[(T_i - T_z)/(C - T_z)] \times 100 = 50$, which is the drug concentration resulting in a 50 % reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in TGI is calculated from $T_i = T_z$. 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 $[(T_i - T_z)/T_z] \times 100 = -50$. 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 lesser than the maximum or minimum concentration tested.

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References

Abadi AH, Eissa AA, Hassan GS (2003) Synthesis of novel 1,3,4-trisubstituted pyrazole derivatives and their evaluation as antitumor and antiangiogenic agents. Chem Pharm Bull 51:838–844

Abbate F, Casini A, Owa T, Scozzafava A, Supura CT (2004) Carbonic anhydrase inhibitors: E7070, a sulfonamide anticancer agent, potently inhibits cytosolic isozymes I and II, and transmembrane, tumor-associated isozyme IX. J Bioorg Med Chem Lett 14:217–223

Abdel-Hamid MK, Abdel-Hafez AA, El-Koussi NA, Mahfouz NA, Innocenti A, Supuran CT (2007) Design, synthesis, and docking studies of new 1,3,4-thiadiazole-2-thione derivatives with carbonic anhydrase inhibitory activity. Bioorg Med Chem 15: 6975–6984

Aboraia AS, Abdel-Rahman HM, Mahfouz NM, EL-Gendy MA (2006) Novel 5-(2-hydroxyphenyl)-3-substituted-2,3-dihydro-1,3,4-oxadiazole-2-thione derivatives: promising anticancer agents. Bioorg Med Chem 14:1236–1246

Akbas E, Beber I (2005) Antibacterial and antifungal activities of new pyrazolo[3,4-*d*]pyridazine derivatives. Eur J Med Chem 40:401–405

Bakhite EA, Mohamed OS, Radwan SM (2002) Synthesis and some reactions of new thieno[2,3-*c*]pyridazine derivatives. Bull Korean Chem Soc 23:1715–1718

Billman HJ, Schmidgall RL (1970) Preparation and antitumor activity of some Schiff bases of 2-amino-4,5-dichlorobenzenesulfonanilid and 2-amino-*p*-toluenesulfonanilide. J Pharm Sci 59:1191–1194

Brana MF, Cacho M, Garcia LM, Mayoral EP, Lopez B, Pascual BDP, Ramos A, Acero N, Llinares F, Munoz D, Lozach O, Meije l (2005) Pyrazolo[3,4-*c*]pyridazines as novel and selective inhibitors of cyclin-dependent kinases. J Med Chem 48:6843–6854

Byth KF, Cooper N, Culshaw JD, Heaton DW, Oakes SEJ, Minshull CA, Norman RA, Pauptil RA, Tucker JA, Breed J, Pannifer A, Rowsell S, Stanway JJ, Valentino APTh (2004) Imidazo[1,2-*b*] pyridazines: a potent and selective class of cyclin-dependent kinase inhibitors. Bioorg Med Chem Lett 14:2249–2252

Combs DW, Rampulla MS, Demers JP, Falotico R, Moore BJ (1992) Heteroatom analogs of bemoradan: chemistry and cardiotonic activity of 1,4-benzothiazinylpyridazinones. J Med Chem 35: 172–176

Curran WV, Ross A (1974) 6-Phenyl-4,5-dihydro-3(2*H*)-pyridazinones. Series of hypotensive agents. J Med Chem 17:273–281

Deeb A, El-Mariah F, Hosny M (2004) Pyridazine derivatives and related compounds. Part 13: synthesis and antimicrobial activity of some pyridazino[3′,4′:3,4]pyrazolo[5,1-*c*]-1,2,4-triazine. Bioorg Med Chem Lett 14:5013–5017

Dilovic I, Rubcic M, Vrdoljak V, Pavelic SK, Kralj M, Piantanida MC (2008) Novel thiosemicarbazone derivatives as potential antitumor agents: synthesis, physicochemical and structural properties, DNA interactions and antiproliferative activity. Bioorg Med Chem 16:5189–5198

Elzbieta W, Prukaia D (1998) New isomeric N-substituted hydrazones of 2-, 3-, and 4-pyridinecarboxyaldehydes. J Heterocycl Chem 35:381–387

Esteves-Souza A, Pissinate K, Nascimento MDG, Greenberg NF, Echevarria A (1998) Synthesis, cytotoxicity, and DNA-topoisomerase inhibitory activity of new asymmetric ureas and thioureas. Bioorg Med Chem 14:492–499

French FA, Blanz EJ, Doamaral JR, French DA (1970) Carcinostatic activity of thiosemicarbazones of formyl heteroaromatic compounds.VI. 1-Formylisoquinoline derivatives bearing additional ring substituent, with notes on mechanism of action. J Med Chem 13:1117–1124

Gerard P, Giovanni P, Paolo D (1986) Conformational behavior and *E*/*Z* isomerization of *N*-acyl and *N*-aroylhydrazones. Tetrahedron 42:3649–3654

Hassall CH, Johnson WH, Theobald CJ (1979) Amino acids and peptides. Part 21. Synthesis of a congener of the cyclohexadepsipeptide antibiotic, monamycin. J Chem Soc Perkin 1:1451–1454

Kamal El-Dean AM, Radwan SM, Abdel Moneam MI (1996) Synthesis and reactivity of some pyridazine derivatives. Afinidid 53:387–391

Kandile NG, Mohamed MI, Zaky H, Mohamed HM (2009) Novel pyridazine derivatives: synthesis and antimicrobial activity evaluation. Eur J Med Chem 44:1989–1996

Khalil AA, Abdel Hamide SG, Al-Obaid AM, El-Subbagh HI (2003) Substituted quinazolines, part 2. Synthesis and in vitro anticancer evaluation of new 2-substituted mercapto-3*H*-quinazoline analogs. Arch Pharm Med Chem 2:95–103

Lewis RT, Blackaby WB, Blackburn T, Jennings ASR, Pike A, Wilson RA, Hallett DJ, Cook SM, Ferris P, Marshall GR, Reynolds DS, Sheppard WFA, Smith AJ, Stanley BSJ, Tye SJ, Wafford KA, Atack JR (2006) A pyridazine series of alpha2/alpha3 subtype selective GABAA agonists for the treatment of anxiety. J Med Chem 49:2600–2610

Loetchutinat C, Chau F, Mankhetkorn S (2003) Synthesis and evaluation of 5-aryl-3-(4-hydroxyphenyl)-1,3,4-oxadiazole-2-(3*H*)-thiones as *p*-glycoprotein inhibitors. Chem Pharm Bull 51:728–730

Malinka W, Redzicka OA (2004) New derivatives of pyrrolo[3,4-*d*] pyridazinone and their anticancer effects. IL Farmaco 59: 457–462

McClusky A, Bowyer MC, Collins E, Sim ATR, Sakoff JA, Baldwin ML (2000) Anhydride modified cantharidin analogues: synthesis, inhibition of protein phosphatases 1 and 2A and anticancer activity. Bioorg Med Chem Lett 10:1687–1690

McEvoy FG, Allen GR (1974) 6-(Substituted phenyl)-5-substituted-4,5-dihydro-3(2*H*)-pyridazinones antihypertensive agents. J Med Chem 17:281–286

Mertens A, Friebe GW, Beckmann BM, Kampe W, Kling L, Saal WVD (1990) Nonsteroidal cardiotonics. 3. New 4,5-dihydro-6-(1*H*-indol-5-yl)pyridazin-3(2*H*)-ones and related compounds with positive inotropic activities. J Med Chem 33:2870–2875

Modi JDS, Sabins S, Deliwala CV (1970) Schiff bases from benzaldehyde nitrogen mustards and aminophenyl-thiazoles. J Med Chem 13:935–941

Monk A, Scudiero DA, Skehan P, Shoemaker RH, Paull KD et al (1991) DTP, DCTD tumor repository a catalog of in vitro cell lines, transplantable animal and human tumors and micro assays. J Natl Cancer Inst 83:757–766

Moreau S, Coudert P, Rubat C, Gardette D, Vallee-Goyet D, Couquelet J, Bastide P, Tronche P (1994) Synthesis and anticonvulsant properties of new benzylpyridazine derivatives. J Med Chem 37:2153–2160

Mylari BL, Armento SJ, Beebe DA, Conn EL, Coutcher J, Dina BMS, Gorman TTO, Linhares MC, Martin WH, Oates PJ, Tess DA, Withbro GJ, Zembrowski WJ (2005) A novel series of non-carboxylic acid, non-hydantoin inhibitors of aldose reductase with potent oral activity in diabetic rat models 6-(5-chloro-3-methylbenzofuran-2-sulfonyl)-2*H*-pyridazin-3-one and congeners. J Med Chem 48:6326–6339

Nagawade RR, Khanna VV, Bhagwat SS, Shinde DV (2005) Synthesis of new series of 1-aryl-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid as potential antibacterial agents. Eur J Med Chem 40:1325–1330

Owa T, Yoshino H, Okauchi T, Yoshimatsu K, Ozawa Y, HataSugi N, Nagasu T, Koyanagi N, Kitoh K (1999) Discovery of novel antitumor sulfonamides targeting G1 phase of the cell cycle. J Med Chem 42:3789–3799

Piaz VD, Giovannoni MP, Castellana C (1997) Novel heterocyclic-fused pyridazinones as potent and selective phosphodiesterase IV inhibitors. J Med Chem 40:1417–1421

Pieretti S, Dominici L, Giannuario AD, Cesari N, Piaz VD (2006) Local anti-inflammatory effect and behavioral studies on new PDE4 inhibitors. Life Sci 79:791–800

Pifferi G, Parravicini F, Carpi C, Dorigotti L (1975) Synthesis and antihypertensive properties of new 3-hydrazinopyridazine derivatives. J Med Chem 18:741–746

Popp FD (1961) Synthesis of potential anticancer agents. II. Some Schiff bases. J Org Chem 26:1566–1568

Popp FD, Kirsch W (1961) Synthesis of potential anticancer agents. V. Schiff bases and related compounds. J Org Chem 26:3858–3860

Quiroga AG, Perez JM, Lopez-Solera IJM, Masagur JR, Luque A, Roman P, Edwards A, Alonso C, Navarro-Ranninger C (1998) Novel tetranuclear orthometalated complexes of Pd(II) and Pt(II) derived from *p*-isopropyl benzaldehyde thiosemicarbazone with cytotoxic activity in cis-DDP resistant tumor cell lines. Interaction of these complexes with DNA. J Med Chem 41:1399–1408

Radwan SM, Bakhite EA (1999) Synthesis of novel thieno[2,3-*c*]pyridazines and related heterocycles. Monatsh Chem 130:1117–1128

Rathish IG, Javed K, Bano S, Ahmad S, Alam MS, Pillai KK (2009) Synthesis and blood glucose lowering effect of novel pyridazinone substituted benzene sulfonylurea derivatives. Eur J Med Chem 44:2673–2678

Rohet F, Rubat C, Coudert P, Couquelet J (1997) Synthesis and analgesic effects of 3-substituted-4,6-diarylpyridazine derivatives of the arylpiperazine class. Bioorg Med Chem 5:655–659

Rostom SAF, Shalaby MA, El-Demellawy MA (2003) Polysubstituted pyrazoles, part 5. Synthesis of new 1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole-3-carboxylic acid hydrazide analogs and some derived ring systems. A novel class of potential antitumor and anti-HCV agents. Eur J Med Chem 38:959–974

Salgin-Goksen U, Gokhan-Kelekci N, Goktas O, Koysal Y, Kilic E, Isak S, Aktay G, Ozalp M (2007) 1-Acylthiosemicarbazides, 1,2,4-triazole-5(4*H*)-thiones, 1,3,4-thiadiazoles and hydrazones containing 5-methyl- 2-benzoxazolinones: synthesis, analgesic, anti-inflammatory and antimicrobial activities. Bioorg Med Chem 15:5738–5751

Schmidt P, Druey J (1954) Heilmittel chemische Studien in der heterocyclischen Reihe. 5. Mitteilung. Pyridazine II. Eine neue Pyridazinsynthese. Helv Chim Acta 37:134–140

Shenvei SL, Brian SE, Xavier TF (F. Hoffman-La Roche). PCT Int Appl Wo2005 105,808-(Cl. c07d495/04). 10 Nov, 2005, US Appl. 2004/PV568, 372, 4 May 2004, Chem. Abst. (2005) 143: 440248x

Takaya M, Sato M, Terashima K, Tanizaw H, Maki Y (1979) A new non-steroidal analgesic-anti inflammatory agent. Synthesis and activity of 4-ethoxy-2-methyl-5-morpholino-3(2*H*)-pyridazinone and related compounds. J Med Chem 22:53–58

Tewari KT, Mishra AJ (2001) Synthesis and anti-inflammatory activities of *N*4, *N*5-disubstituted-3-methyl-1*H*-pyrazolo[3,4-*c*] pyridazines. Bioorg Med Chem 9:715–718

Tomil MC, Estevez I, Ravina E, Orallo F (1998) Study of the vasorelaxant action of 6-aryl-5-piperidino-3-hydrazinopyridazines in isolated rat thoracic aorta: comparison with hydralazine. Gen Pharmacol 30:201–207

Urban M, Sarek J, Klinot J, Korinkova G, Hajduch M (2004) Synthesis of A-seco derivatives of betulinic acid with cytotoxic activity. J Nat Prod 67:1100–1105

Wermuth CG, Schlewer G, Bourguignon JJ, Maghioros G, Bouchet MJ, Moire C, Kan JP, Worms P, Biziere K (1989) 3-Aminopyridazine derivatives with atypical antidepressant, serotonergic and dopaminergic activities. J Med Chem 32:528–537

Wu J, Song B, Chen H, Bhadury P, Hu D (2009) Synthesis and antifungal activity of 5-chloro-6-phenyl-pyridazin-3(2*H*)-one derivatives. Molecules 14:3676–3687

Yamaguchi M, Maruyama N, Koga T, Kamel K, Akima M, Kuroki T, Hamana M, Ohi N (1995) Novel antiasthmatic agents with dual activities of thromboxane A2 synthetase inhibition and bronchodilation. V. Thienopyridazinone derivatives. Chem Pharm Bull 43:236–240