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Full Paper

Synthesis and Anticancer Activities of Novel 1,4-Disubstituted Phthalazines

Juan Li [†], Yan-Fang Zhao [‡], Xiao-Ye Yuan, Jing-Xiong Xu and Ping Gong *

School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenhe District, 110016 Shenyang, Liaoning, P. R. China; Fax: (+86) 24-23882925/23986426, Phone: (+86) 24-23986426; E-mails: [†] lijuan107628@sian.com; [‡] yanfangzhao@126.com

* Author to whom correspondence should be addressed; E-mail: gongpinggp@126.com or gongping@syphu.edu.cn

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Abstract: A series of novel 1-anilino-4-(arylsulfanylmethyl)phthalazines were designed and synthesized. The structures of all the compounds were confirmed by IR, ^{1}H-NMR, elemental analysis and MS. The analogues 1-(3-chloro-4-fluoroanilino)-4-(3,4-difluorophenylthio-methyl)phthalazine (**12**) and 1-(4-fluoro-3-trifluoromethylanilino)-4-(3,4-difluorophenyl-thiomethyl)phthalazine (**13**) showed higher activity than a cisplatin control when tested *in vitro* against two different cancer cell lines using the microculture tetrazolium method (MTT) method.

Keywords: Phthalazine derivatives, synthesis, anticancer activities.

Introduction

Phthalazine derivatives, like the other members of the isomeric benzodiazine series, have been widely applied as therapeutic agents due to their anticonvulsant, cardiotonic, vasorelaxant and anti-inflammatory properties [1,2]. To our knowledge, however, there have been no reports on the anticancer activities of 1-anilino-4-arylsulfanylmethylphthalazines. We describe here the synthesis of some novel 1-anilino-4-arylsulfanylphthalazine derivatives **9-19**, several of which exhibited higher activity than the cisplatin control.

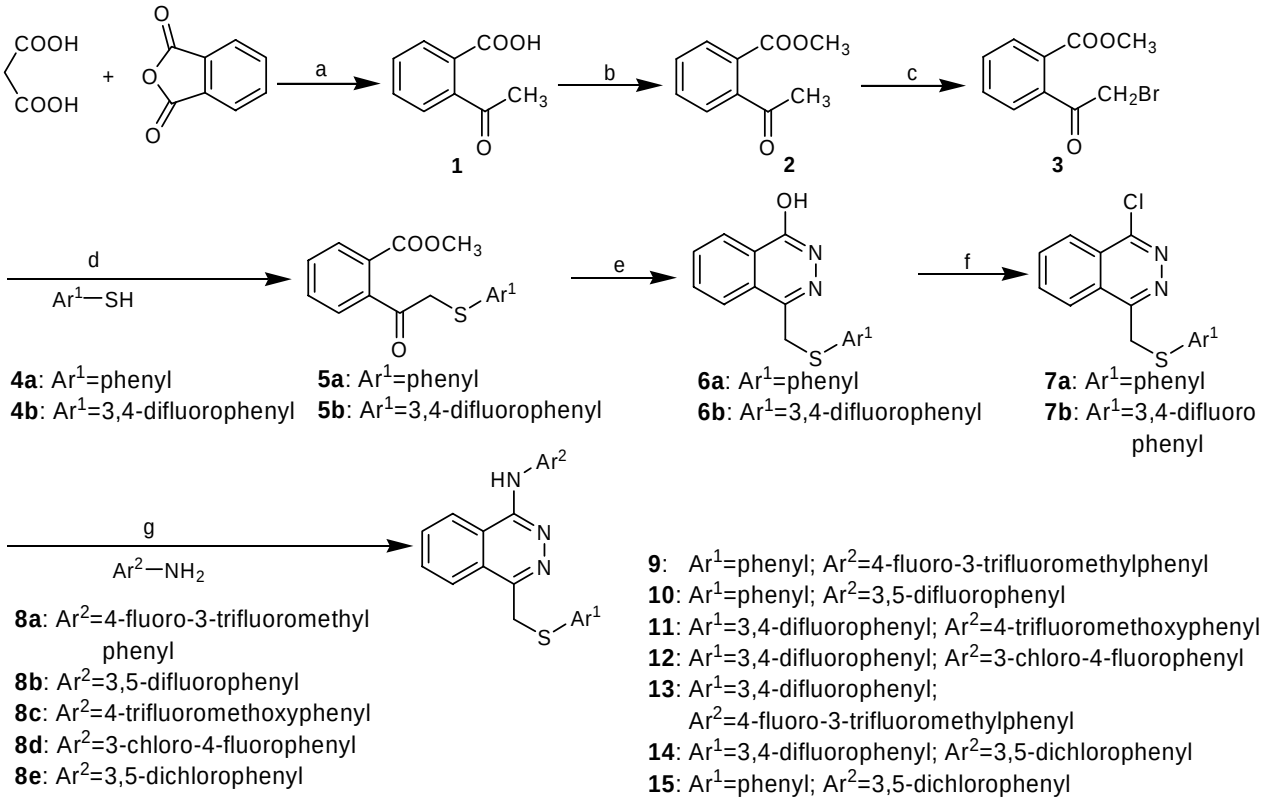
Phthalazines were previously synthesized from 2-aryl-3-hydroxyinden-1-ones or *β*-diketones by condensation with hydrazine hydrate [2-5]. However, these routes do not allow for the desired incorporation of thiophenylmethyl groups into the phthalazine 4-position. This report describes a convenient access to 1,4-disubstituted phthalazines with such substituents. The compounds obtained in were characterized by IR, ^{1}H-NMR, MS and elemental analysis and their anticancer activities were evaluated *in vitro*.

Results and Discussion

Chemistry

Our syntheses of the requisite phthalazines are illustrated in Schemes 1 and 2. Refluxing phthalic anhydride and malonic acid in pyridine gave 2-acetylbenzoic acid (**1**) [6], which was then esterified with dimethyl sulfate. In the next step, the acetyl group was brominated with phenyltrimethyl-ammonium tribromide (PTT), a selective brominating reagent for ketones or ketals [7], to give intermediate **3**, which facilitated the introduction of various thiophenol substituents. It should be noted that side-products could be formed if bromine was used as the halogen source. Compound **3** was then treated with thiophenol or 3,4-difluorothiophenol using K_2CO_3 as base. Cyclization of **5a/5b** with hydrazine hydrate led to the generation in 90-96% yields of the phthalazines **6a/6b**, which were treated with $POCl_3$ to give 1-chloro-4-substituted-phthalazines **7a** and **7b**. Treatment of **7a/7b** with substituted anilines provided the target compounds **9-15**.

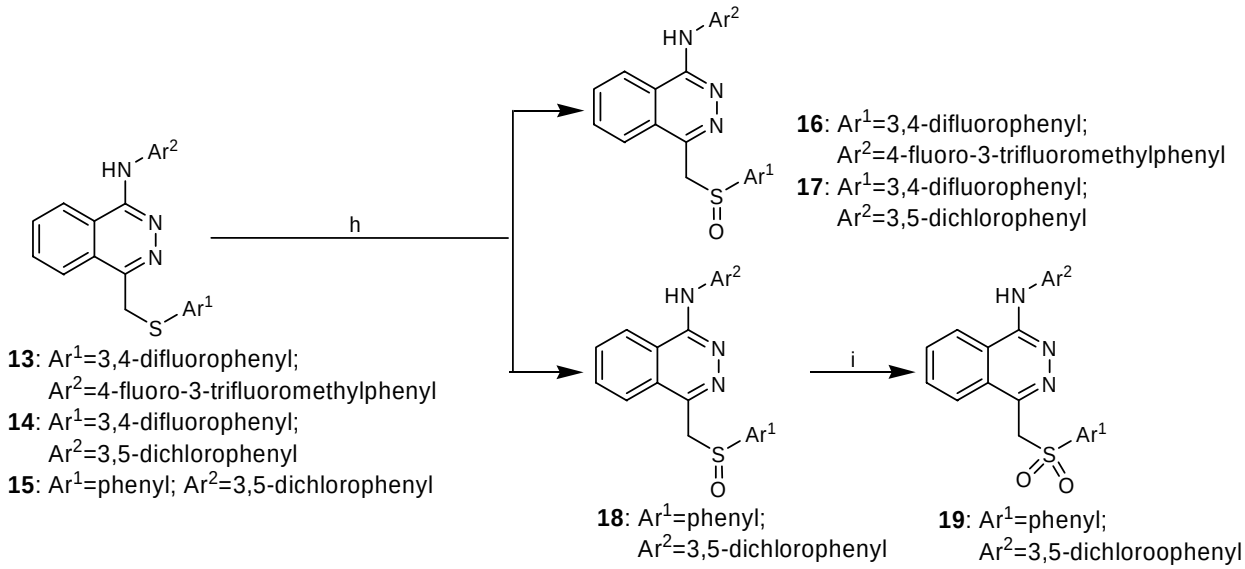
Scheme 1. Synthesis of compounds **9-15**.



Reagents and conditions: a) Py, reflux, 3 h; b) Me_2SO_4, K_2CO_3, acetone, reflux, 3 h; c) PTT, THF, r.t., 15 h; d) K_2CO_3, CH_3OH, r.t, 1.5 h; e) $H_2NNH_2 \cdot H_2O$, CH_3OH, reflux, 5 h; f) $POCl_3$, 110 ^oC, 3 h; g) *i*-PrOH, 50 ^oC, 3 h

The target compounds **16-18** could be obtained by oxidization of the corresponding compounds **13-15** with H_2O_2. However, the sulfanyl substituted phthalazine derivatives could not be easily converted to the corresponding sulfonyl ones. MCPBA has been used as the oxidizing reagent in some cases [8], but in our synthetic route this could easily lead to an undesired N-oxidized side-product, due to the presence of the amino groups. We have found, however, that H_2O_2/Na_2WO_4 was very effective for oxidizing sulfanyl to sulfonyl groups in good yield and high purity.

Scheme 2. Synthesis of compounds **16-19.**



Reagents and conditions: h) H_2O_2, HOAc, r.t., 16 h; i) $Na_2WO_4{\cdot}2H_2O$, H_2O_2, CH_3OH, r.t., 18 h

Anticancer activities

The anticancer activities of compounds **9-19** were evaluated *in vitro* by the MTT method and the results are summarized in Table 1.

Table 1. The anticancer activities of compounds **9-19.**

Compd.	IC_{50} (μM)		Compd.	IC_{50} (μM)	
	Bel-7402	HT-1080		Bel-7402	HT-1080
9	146.8	83.9	**15**	91.3	126.5
10	116.1	163.4	**16**	164.2	122.4
11	56.2	38.9	**17**	125.0	133.6
12	32.4	25.4	**18**	198.6	158.4
13	30.1	25.8	**19**	244.0	169.9
14	69.2	60.3	cisplatin	73.3	63.3

Compounds **12** and **13** showed more *in vitro* activity than cisplatin against the two cancer cell lines tested. The phthalazine derivative **11** showed activity comparable to cisplatin. The remaining compounds exhibited slight to moderate activities.

Conclusions

A series of novel 1,4-disubstituted phthalazines have been prepared. From the biological test results the following conclusions can be reached about their structure-activity relationships: (a) incorporation of a substituted thiophenol group into position 4 of the phthalazine ring appears to increase anticancer activity, compared to that of an unsubstituted thiophenol; (b) replacement of a sulfanyl with sulfinyl or sulfonyl groups decreases the anticancer activity. Further investigations are in process.

Experimental

General

Melting points were determined by the capillary tube method, and the thermometer was uncorrected. Mass spectra were obtained on an Agilent 1100 HPLC-MS instrument. ^{1}H-NMR spectra were run in DMSO-d_6, with TMS at the internal standard, on a Bruker ARX-300 instrument operating at 300 MHz. IR spectra (KBr disks) were recorded on a Bruker IFS 55 instrument. Elemental analysis was performed with a Carlo-Erba 1106 Elemental analysis instrument.

Chemistry

2-Acetylbenzoic acid (**1**)

A mixture of phthalic anhydride (22.2 g, 0.15 mol), malonic acid (18.7 g, 0.18 mol) and pyridine (17.3 mL, 0.18 mol) was refluxed for 3 h. The resulting mixture was then cooled to 30 ^{o}C, water (160 mL) was added and the mixture was stirred for 30 min. The insoluble material was filtered off and the filtrate was treated with concentrated HCl to pH 3-4. Filtration and recrystallization from chloroform gave 16.8 g (68%) of **1**, m.p. 113-114 ^{o}C (lit. [6] 114-115 ^{o}C); MS: *m/z* 165 (MH^+); IR (cm^{-1}): 3450.1 (OH), 1685.2, 1676.3 (C=O), 1639.7, 1590.6 (C=C).

Methyl 2-acetylbenzoate (**2**)

A mixture of 2-acetylbenzoic acid (**1**, 100.0 g, 0.61 mol), dimethyl sulfate (92.0 g, 0.73 mol) and K_2CO_3 (50.0 g, 0.37 mol) was refluxed for 3 h in acetone. The reaction mixture was cooled to room temperature and filtered. The filtrate was distillated under reduced pressure collecting the fraction with b.p. 86-92 ^{o}C/6 mmHg, which gave product **2** as a light yellow oil, 84 g (77%); GC purity: 97.6%; MS: *m/z* 179 (MH^+).

Methyl 2-(bromocarbonyl)benzoate (**3**)

To a solution of **2** (100.0 g, 0.56 mol) in anhydrous tetrahydrofuran (200 mL), a solution of PTT (210.5 g, 0.56 mol) in anhydrous tetrahydrofuran (80 mL) was added dropwise. During this addition a

white precipitate was formed and the solution became yellow. After stirring at room temperature for 15 h, the resulting mixture was filtered. The filtrate was stirred into a mixture of petroleum ether/water (200 mL, 1:1 v/v), then separated and concentrated to give 116.4 g (80%) of **3**; m.p.132-133 ^{o}C; MS: *m/z* 257, 259 (MH^+); IR (cm^{-1}): 2938.3 (CH), 1689.2, 1681.3 (C=O), 1647.7, 1602.6 (C=C); Anal. Calcd. for $C_{10}H_9BrO_3$: C 46.72, H 3.53, Br 31.08; Found: C 46.60, H 3.34, Br 31.19.

Methyl 2-(2-(phenylthio)acetyl)benzoate (**5a**)

To a mixture of K_2CO_3 (8.3 g, 0.06 mol) and **4a** (11 g, 0.1 mol) in methanol (150 mL), a solution of **3** (25.7 g, 0.1 mol) in acetone (200 mL) was added dropwise while the temperature was kept below 0 ^{o}C. The reaction mixture was stirred for an additional 1.5 h at this temperature. After filtration and concentration, the residue was dissolved in dichloromethane (200 mL). The organic phase was washed with saturated sodium carbonate solution (100 mL×3) and dried with $MgSO_4$. Concentration gave **5a** as an oil, 23.5 g (82%, GC purity: 96.9%), which could be used in next step without purification.

Methyl 2-(2-(3,4-difluorophenylthio)acetyl)benzoate (**5b**).

Prepared using **4b** as described for **5a**; yellow oil, 27.5 g, (86%), GC purity: 98.3%.

1-Hydroxy-4-phenylthiomethylphthalazine (**6a**)

Hydrazine hydrate (15.6 g, 80%, 0.25 mol) was added into a solution of **5a** (24.0 g, 84 mmol) in methanol (100 mL). The mixture was refluxed for 5 h. After cooling to room temperature, a solid mass precipitated. Filtration and recrystallization from ethyl acetate gave **6a**, 20.4 g, (91%), m.p. 146-148 ^{o}C; MS: *m/z* 269 (MH^+); IR (cm^{-1}): 3610.3 (OH), 1611.1, 1549.5 (C=C); ^{1}H-NMR: δ 4.89 (s, 2H, CH_2), 7.27-7.31 (m, 3H, Ph-3H), 7.42 (d, *J*=7.0 Hz, 2H, Ph-2H), 8.17-8.25 (m, 2H, phthalazinyl-2H), 8.42 (d, *J*=8.0 Hz, 1H, phthalazinyl-H), 8.79 (d, *J*=8.0 Hz, 1H, phthalazinyl-H).

1-Hydroxy-4-(3,4-difluorophenyl)thiomethylphthalazine (**6b**).

Prepared using **5b** as described for **6a**, 24.2 g, (95%), m.p. 163-164 ^{o}C; MS: *m/z* 305 (MH^+); IR (cm^{-1}): 3408.3 (OH), 1613.1, 1567.3, 1509.6 (C=C); ^{1}H-NMR: δ 4.85 (s, 2H, CH_2), 7.23 (b, 1H, Ph-H), 7.59-7.67 (m, 2H, Ph-2H), 8.20-8.27 (m, 2H, phthalazinyl-2H), 8.47-8.49 (d, *J*=8.1 Hz, 1H, phthalazinyl-H), 9.04-9.05 (d, d, *J*=8.1 Hz, 1H, phthalazinyl-H).

1-Chloro-4-phenylthiomethylphthalazine (**7a**)

Phosphorus oxychloride (36.8 g, 0.24 mol) was added dropwise into a solution of **6a** (104.5 g, 0.39 mol) in pyridine (37 mL, 0.47 mol). The mixture was slowly heated to 110 ^{o}C and stirred for 1h. After cooling to 50 ^{o}C, chloroform (100 mL) and cold water (100 mL) were added. The biphasic mixture was stirred for 30 min and the layers were separated. The organic layer was washed with 5% sodium bicarbonate solution, dried and concentrated. The residue was triturated with diethyl ether by stirring

for 3 h to give a suspension that was filtered to afford **7a**, 92.8 g, (83%), m.p. 151-153 ^{o}C; MS: *m/z* 285, 287 (MH^+); IR (cm^{-1}): 1612.1, 1550.6 (C=C); ^{1}H-NMR: δ 4.85 (s, 2H, CH_2), 7.27-7.30 (m, 3H, Ph-3H), 7.44 (d, *J*=6.9 Hz, 2H, Ph-2H), 8.27-8.36 (m, 2H, phthalazinyl-2H), 8.63 (d, *J*=7.9 Hz, 1H, phthalazinyl-H), 8.79 (d, *J*=7.9 Hz,1H, phthalazinyl-H).

1-Chloro-4-phenylthiomethylphthalazine (**7b**).

Prepared using **6b** as described for **7a**, 105.7 g, (84%), m.p. 173-175 ^{o}C; MS: *m/z* 321, 323 (MH^+); IR (cm^{-1}): 1619.2, 1583.6, 1512.1 (C=C); ^{1}H-NMR: δ 4.84 (s, 2H, CH_2), 7.25 (b, 1H, Ph-H), 7.55-7.64 (m, 2H, Ph-2H), 8.29-8.37 (m, 2H, phthalazinyl-2H), 8.72 (d, *J*=7.9 Hz, 1H, phthalazinyl-H), 8.68 (d, *J*=7.9 Hz,1H, phthalazinyl-H).

1-(4-Fluoro-3-trifluoromethylanilino)-4-phenylthiomethylphthalazine (**9**)

A mixture of **7a** (0.86 g, 3 mmol) and **8a** (0.72 g, 4 mmol) in isopropanol (20 mL) was heated to 50 ^{o}C for 3 h, then the mixture was concentrated *in vacuo*. The resulting red oil was triturated with diethyl ether (30 mL), by stirring for 10 min to give a suspension. Filtration and recrystallization from ethyl acetate/cyclohexane yielded 1.0 g (81%) of **9**, m.p. 214-215 ^{o}C; MS: *m/z* 430 (MH^+); IR (cm^{-1}): 3440.3 (NH), 1613.7, 1556.6, 1506.9 (C=C); ^{1}H-NMR: δ 4.84 (s, 2H, CH_2), 7.25-7.31 (m, 3H, Ar_1-3H), 7.40 (d, *J*=6.9 Hz, 2H, Ar_1-2H), 7.69 (t, *J*=6.8 Hz, 1H, Ar_2-H), 7.97 (m, 1H, Ar_2-H), 8.14 (m, 1H, Ar_2-H), 8.28 (m, 2H, phthalazinyl-2H), 8.52 (m, 1H, phthalazinyl-H), 8.96 (m, 1H, phthalazinyl-H); Anal. Calcd. for $C_{22}H_{15}F_4N_3S$: C 61.53, H 3.52, N 9.79; Found: C 61.42, H 3.41, N 9.70.

1-(3,5-Difluoroanilino)-4-phenylthiomethylphthalazine (**10**)

Prepared using **7a** and **8b** as described for **9**, 0.97 g, (85%), m.p. 198-199 ^{o}C; MS: *m/z* 380 (MH^+); IR (cm^{-1}): 3441.8 (NH), 1625.8, 1553.9, 1478.6 (C=C); ^{1}H-NMR: δ 4.88 (s, 2H, CH_2), 7.12 (b, 1H, Ar_2-H), 7.26 (t, *J* 7.4 Hz, 1H, Ar_1-H), 7.32 (t, *J*=7.5 Hz, 2H, Ar_1-2H), 7.40 (d, *J*=7.5 Hz, 2H, Ar_1-2H), 7.55 (d, *J*=7.8 Hz, 2H, Ar_2-2H), 8.20-8.26 (m, 2H, phthalazinyl-2H), 8.50 (d, *J*=8.0 Hz, 1H, phthalazinyl-H), 8.95 (d, *J* 8.0 Hz, 1H, phthalazinyl-H); Anal. Calcd. for $C_{21}H_{15}F_2N_3S$: C 66.48, H 3.98, N 11.07; Found: C 66.52, H 3.79, N 11.12.

1-(4-Trifluoromethoxyanilino)-4-(3,4-difluorophenylthiomethyl)phthalazine (**11**)

Prepared using **7b** and **8c** as described for **9**, 1.2 g, (84%), m.p. 219-221 ^{o}C; MS: *m/z* 464 (MH^+); IR (cm^{-1}): 3465.6 (NH), 1601.7, 1548.0, 1506.6 (C=C); ^{1}H-NMR: δ 4.85 (s, 2H, CH_2), 7.23 (m, 1H, Ar_2-H), 7.33-7.42 (q, *J*=8.8 Hz, 1H, Ar_2-H), 7.52 (d, *J*=8.6 Hz, 2H, Ar_2-2H), 7.60-7.67 (m, 1H, Ar_1-H), 7.75 (d, *J*=8.6 Hz, 2H, Ar_1-2H), 8.22-8.26 (m, 2H, phthalazinyl-2H), 8.47-8.49 (b, 1H, phthalazinyl-H), 9.04-9.05 (b, 1H, phthalazinyl-H); Anal. Calcd. for $C_{22}H_{14}F_5N_3OS$: C 57.02, H 3.04, N 9.07; Found: C 57.13, H 3.15, N 9.10.

1-(3-Chloro-4-fluoroanilino)-4-(3,4-difluorophenylthiomethyl)phthalazine (**12**)

Prepared using **7b** and **8d** as described for **9**, 1.1 g, (87%), m.p. 208-210 ^{o}C; MS: *m/z* 432 (M^+); IR (cm^{-1}): 3442.1 (NH), 1616.3, 1565.4, 1505.0 (C=C); ^{1}H-NMR: δ 4.86 (s, 2H, CH_2), 7.12 (b, 1H, Ar_2-H), 7.36-7.41 (q, *J*=8.6 Hz, 1H, Ar_1-H), 7.59 (t, *J*=9.0 Hz, 1H, Ar_1-H), 7.62-7.65 (m, 2H, Ar_2-2H), 7.96 (d, *J*=5.2 Hz, 1H, Ar_1-H), 8.21-8.26 (m, 2H, phthalazinyl-2H), 8.48 (d, *J*=7.4 Hz, 1H, phthalazinyl-H), 9.02 (d, *J*=7.3 Hz, 1H, phthalazinyl-H); Anal. Calcd. for $C_{21}H_{13}ClF_2N_3S$: C 58.40, H 3.03, N 9.73; Found: C 58.40, H 2.89, N 9.58.

1-(4-Fluoro-3-trifluoromethylanilino)-4-(3,4-difluorophenylthiomethyl)phthalazine (**13**)

Prepared using **7b** and **8a** as described for **9**, 1.1 g, 80%, m.p. 220-222 ^{o}C; MS: *m/z* 466 (MH^+); IR (cm^{-1}): 3447.2 (NH), 1604.2, 1564.8, 1503.5 (C=C); ^{1}H-NMR: δ 4.86 (s, 2H, CH_2), 7.21-7.23 (b, 1H, Ar_1-H), 7.35-7.42 (q, *J*=8.5 Hz, Ar_2-H), 7.59-7.70 (q, *J*=8.5 Hz, 2H, Ar_1-2H), 7.99 (m, 1H, Ar_2-H), 8.17 (d, *J*=7.8 Hz, 1H, Ar_2-H), 8.21-8.25 (m, 2H, phthalazinyl-2H), 8.47-8.50 (m, 1H, phthalazinyl-H), 8.97 (b, 1H, phthalazinyl-H); Anal. Calcd. for $C_{22}H_{13}F_6N_3S$: C 56.77, H 2.82, N 9.03; Found: C 56.61, H 2.90, N 8.98.

1-(3,5-Dichloroanilino)-4-(3,4-difluorophenylthiomethyl)phthalazine (**14**)

Prepared using **7b** and **8e** as described for **9**, 1.0 g, 87%, m.p. 233-234 ^{o}C; MS: *m/z* 448 (M^+); IR (cm^{-1}): 3437.8 (NH), 1605.7, 1571.7, 1502.1 (C=C); ^{1}H-NMR: δ 4.88 (s, 2H, CH_2), 7.22 (d, *J* 8.6 Hz, 1H, Ar_1-H), 7.36-7.40 (q, *J*=8.6 Hz, 1H, Ar_1-H), 7.47 (s, 1H, Ar_2-H), 7.63 (t, *J*=8.2 Hz, 1H, Ar_1-H), 7.89 (s, 2H, Ar_2-2H), 8.19-8.24 (m, 2H, phthalazinyl-2H), 8.48 (d, *J*=7.8 Hz, 1H, phthalazinyl-H), 8.90 (d, *J*=7.8 Hz, 1H, phthalazinyl-H); Anal. Calcd. for $C_{21}H_{13}Cl_2F_2N_3S$: C 56.26, H 2.92, N 9.37; Found: C 56.37, H 2.83, N 9.38.

1-(4-Trifluoromethoxyanilino)-4-phenylthiomethyl)phthalazine (**15**)

Prepared using **7a** and **8c** as described for **9**, 1.1 g, 85%, m.p. 193-195 ^{o}C; MS: *m/z* 428 (MH^+); IR (cm^{-1}): 3447.9 (NH), 1619.0, 1555.8, 1508.2 (C=C); ^{1}H-NMR: δ 4.84 (s, 2H, CH_2), 7.24-7.33 (m, 3H, Ar_1-3H), 7.40 (d, *J*=7.7 Hz, 2H, Ar_1-H), 7.50 (d, *J*=8.5 Hz, 2H, Ar_2-2H), 7.77 (d, *J*=8.5 Hz, 2H, Ar_2-2H), 8.23-8.26 (m, 2H, phthalazinyl-2H), 8.49 (d, *J*=8.0 Hz, 1H, phthalazinyl-H), 9.05 (d, *J*=8.0 Hz, 1H, phthalazinyl-H); Anal. Calcd. for $C_{22}H_{16}F_3N_3OS$: C 61.28, H 3.77, N 9.83; Found: C 61.99, H 3.56, N 9.63.

1-(4-Fluoro-3-trifluoromethylanilino)-4-(3,4-difluorophenylsulfinylmethyl)phthalazine (**16**)

30% aqueous H_2O_2 (2.2 g, 19 mmol) was added into a solution of **13** (6.0 g, 13 mmol) in acetic acid (10 mL).The reaction mixture was stirred for 16 h at room temperature. It was then poured into water and neutralized with 5% NaOH. Filtration and recrystallization from ethyl acetate/chloroform gave 5.7 g (91%) of **16**, m.p. 233-234 ^{o}C; MS: *m/z* 482 (MH^+); IR (cm^{-1}): 3355.2 (NH), 1574.4, 1505.2,

(C=C); ^{1}H-NMR: δ 4.78-4.99 (q, *J*=13.2 Hz, 2H, CH_2), 7.49-7.63 (m, 3H, Ar_1-3H), 7.77 (t, *J*=9.0 Hz, 1H, Ar_2-H), 8.03 (m, 2H, Ar_2-2H), 8.25 (s, 2H, phthalazinyl-2H), 8.44 (d, *J*=8.1 Hz, 1H, phthalazinyl-H), 8.57 (d, *J*=8.1 Hz, 1H, phthalazinyl-H); Anal. Calcd. for $C_{22}H_{13}F_6N_3OS$: C 54.89, H 2.72, N 8.73; Found: C 55.03, H 2.93, N 8.95.

1-(3,5-Dichloroanilino)-4-(3,4-difluorophenylsulfinylmethyl)phthalazine (**17**)

Prepared using **14** as described for **16**, 5.8 g, (96%), m.p. 241-242 ^{o}C. MS: *m/z* 465 (MH^+); IR (cm^{-1}): 3425.9 (NH), 1592.4, 1575.8, 1502.5 (C=C); ^{1}H-NMR: δ 4.83-5.01 (q, *J*=6.7 Hz, 2H, CH_2), 7.22 (s, 1H, Ar_2-H), 7.46 (d, *J*=6.1 Hz, 1H, Ar_1-H), 7.59-7.63 (q, *J*=8.4 Hz, 1H, Ar_1-H), 7.77 (t, *J*=7.9 Hz, 1H, Ar_1-H), 7.99 (s, 2H, Ar_2-2H), 8.10 (s, 2H, phthalazinyl-2H), 8.33 (d, *J*=8.0 Hz, 1H, phthalazinyl-H), 8.58 (d, *J* 8.0 Hz, 1H, phthalazinyl-H); Anal. Calcd. for $C_{21}H_{13}Cl_2F_2N_3OS$: C 54.32, H 2.82, N 9.05; Found: C 54.29, H 2.73, N 9.16.

1-(4-Trifluoromethoxyanilino)-4-phenylsulfinylmethylphthalazine (**18**)

Prepared using **15** as described for **16**, 5.0 g, 91%, m.p. 225-226 ^{o}C. MS: *m/z* 466 ($M+Na^+$); IR (cm^{-1}): 3438.5 (NH), 1637.2, 1560.6, 1509.6 (C=C); ^{1}H-NMR: δ 4.79-4.83 (q, *J*=6.8 Hz, 2H, CH_2), 7.34 (d, *J*=8.9 Hz, 2H, Ar_2-2H), 7.50-7.53 (m, 3H, Ar_1-3H), 7.64 (d, *J*=7.7 Hz, 2H, Ar_1-2H), 7.99 (d, *J* =8.0 Hz, 2H, Ar_2-2H), 8.05 (s, 2H, phthalazinyl-2H), 8.21 (d, *J*=7.9 Hz, 1H, phthalazinyl-H), 8.63 (d, *J* =7.9 Hz, 1H, phthalazinyl-H); Anal. Calcd. for $C_{22}H_{16}F_3N_3O_2S$: C 59.59, H 3.64, N 9.48; Found: C 59.66, H 3.90, N 9.67.

1-(4-Trifluoromethoxyanilino)-4-phenylsulfonylmethylphthalazine (**19**)

$NaWO_4 \cdot 2H_2O$ (2.5 mmol) and 30% aqueous H_2O_2 (11.3 g, 100 mmol) were added into a solution of **15** (4.5 g, 10.6 mmol) in methanol (20 mL). The mixture was stirred at room temperature for 18 h, filtered and washed with water. Recrystallization from ethyl acetate/acetone afforded **19** as off-white powder, 4.1 g (84%), m.p. 228-229 ^{o}C; MS: *m/z* 460 (MH^+); IR (cm^{-1}): 3416.7 (NH), 1622.1, 1566.6, 1508.8 (C=C); ^{1}H-NMR: δ 5.33 (s, 2H, CH_2), 7.37 (d, *J*=8.5 Hz, 2H, Ar_2-2H), 7.58 (t, *J*=7.4 Hz, 2H, Ar_1-2H), 7.69-7.76 (m, 3H, Ar_1-3H), 7.96 (d, *J*=8.5 Hz, 2H, Ar_2-2H), 7.99 (s, 2H, phthalazinyl-2H), 8.27 (d, *J*=8.0 Hz, 1H, phthalazinyl-H), 8.60 (d, *J*=8.1 Hz, 1H, phthalazinyl-H); Anal. Calcd. for $C_{22}H_{16}F_3N_3O_3S$: C 57.51, H 3.51, N 9.15; Found: C 57.20, H 3.34, N 8.99.

Pharmacology

The anticancer activities of compounds **9-19** were evaluated *in vitro* on Bel-7402 (Human Liver Cancer cell lines) and HT-1080 (Human Fibro Sarcoma cell lines) by measuring cell viability by the MTT method, with cisplatin as the positive control. The cells were seeded in RPM I 1640 medium (100 µL) in a 96-well plate at a concentration of 4000 cells per well. After culturing for 12 h at 37 ^{o}C and 5% CO_2, cells were incubated with various concentrations of the samples for 24 h. MTT was added at a terminal concentration of 5µg/mL and incubated with the cells for 4 h. The formazan crystals were dissolved in DMSO (100 µL) in each well and the optical density was measured at 492

nm (for the absorbance of MTT formazan) and 630 nm (for the reference wavelength). The IC_{50} was calculated using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

References

1. Tsoungas, P. G.; Searcey, M. A convenient access to benzo-substituted phthalazines as potential precursors to DNA intercalators. *Tetrahedron Lett.* **2001**, *42*, 6589-6592.
2. Sivakumar, R.; Gnanasam, S. K.; Ramachandran, S.; Leonard, J. T. Pharmacological evaluation of some new 1-substituted-4-hydroxyphthalazines. *Eur. J. Med. Chem.* **2002**, *37*, 793-801.
3. Napoletano, M.; Norcini, G.; Pellacini, F.; Marchini, F.; Morazzoni, G.; Ferlenga, P.; Pradella, L. Phthalazine PDE4 Inhibitors. Part 2: the synthesis and biological evaluation of 6-methoxy-1,4-disubstituted derivatives. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 33-37.
4. Haack, T.; Fattori, R.; Napoletano, M.; Pellacini, F.; Fronza, G.; Raffaini, G.; Ganazzoli, F. Phthalazine PDE IV inhibitors: conformational study of some 6-methoxy-1,4-disubstituted derivatives. *Bioorg. Med. Chem.* **2005**, *13*, 4425-4433.
5. Joule, J. A.; Smith, G. F. Synthesis of diazine rings. *Hetercycl. Chem.* **1978**, 138-139.
6. Yale, H. L. *o*-Acetobenzoic acid, its preparation and lactonization. A novel application of the Doebner synthesis. *J. Amer. Chem. Soc.* **1947**, *69*, 1547-1548.
7. Jaques, J.; Marquet, A. Selective α-bromination of an aralkyl ketone with phenyltrimethyl-ammonium tribromide: 2-bromoacetyl-6-methoxynaphthalene and 2,2-dibromoacetyl-6-methoxy-naphthalene. *Org. Syn.* **1976**, *53*, 111.
8. André, B. C.; Carl, B.; David S. M. An expedient approach to *E,Z*-dienes using the Julia olefination. *Tetrahedron Lett.* **2001**, *42*, 5149-5153.

Sample availability: Samples of the compounds mentioned are available from the corresponding author.