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Synthesis and antitumor activity of *s*-tetrazine derivatives

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Abstract—Fifty-five compounds of *s*-tetrazine derivative including hexahydro-, 1,6-dihydro, 1,4-dihydro-, 1,2-dihydro- and aromatic *s*-tetrazine were prepared. Their antitumor activities were evaluated in vitro by MTT method for P-388 cell and SRB method for A-549 cell. The results show that there are 9 compounds which in 10^{-6} μM have more than 50% inhibition rate to A-549 cancer cell growth, and 7 compounds in 10^{-6} μM have more than 50% inhibition rate to P-388 cancer cell growth. The IC_{50} of compound **3q** for P-388, Bel-7402, MCF-7 and A-549 are 0.6 μM, 0.6 μM, 0.5 μM and 0.7 μM, respectively. So *s*-tetrazine derivative is a kind of compound which possesses potential antitumor activities and is worth to research further.

There are several reviews[1] indicating that the use of compounds containing the 1,2,4,5-tetrazine skeleton had been claimed for use as pharmaceuticals. For example, 3-amino-6-aryl-1,2,4,5-tetrazines showed modest antimalarial activity,[2] some hexahydro-*s*-tetrazines proved to have useful analgesic and antiflammatory activity.[3] For a series of tetrahydro-*s*-tetrazines the antibacterial and antifungal activities have been evaluated,[4] a special 1,4-dihydro-*s*-tetrazine derivative was pronounced antiviral activity.[1b]

Among them, the hexahydro-*s*-tetrazine (Fig. 1) was recommended as an antitumor agent.[5] Although there was not any data about antitumor activities to be reported, it is the first time to announce that this kind of compound may possess potential antitumor activity. We are interested in whether changing the structure is possible to improve the antitumor activity or not.

Fifty-five compounds† including hexahydro- (**1**), 1,6-dihydro- (**2**), 1,4-dihydro- (**3**), 1,2-dihydro- (**4**) and aromatic (**5**) *s*-tetrazines were prepared according literature methods.[6] The routes of synthesis are shown in Scheme 1 and Scheme 2. The results were summarized in Table 1. When preparing **2a–b** from **1a–c**, the Skorianetz method[6c] was modified with using of cheaper Pd/C catalyst instead of PtO_2.[7] The **3a–z** were prepared by the new reaction of **2a–b** with substituted phenyl isocyanate under 4-dimethylaminopyridine (DMAP) as catalyst.[8] The stereostructure of **3d** was determined by X-ray analysis as shown in Figure 2, [9] which clearly shows the *N*,*N*′-substituents are at the 1,4-positions as we expected. Therefore, it proved that **3** are 1,4-dihydro-*s*-tetrazine derivatives and that a rearrangement occurs in the reaction. And the structure of **4c** was determined by X-ray analysis as shown in Figure 3,[10] which clearly shows *N*,*N*′-substituents are at the 1,2-positions, thus proving that **4** are 1,2-dihydro-*s*-tetrazine derivatives.

The antitumor activities in vitro for these compounds were evaluated by method MTT for P-388 cell and SRB for A-549. The results were summarized in Table 2.

Usually, when the concentration of the compound solution is 10^{-6} mol/L, the inhibition ratio of the solution to cancer cell growth is more than 50%, the compound is considered as a strong effective. According this standard, it can be found from Table 2 that hexahydro-*s*-tetrazine (**1a–g**), 1,6-dihydro-*s*-tetrazine (**2a–m**), 1,2-dihydro-*s*-tetrazine (**4a–g**) and aromatic *s*-tetrazine (**5a–j**) have almost no activities except **4f** which has strong

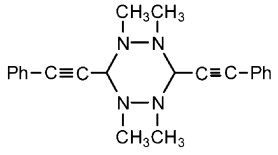

Figure 1.

Keywords: Synthesis; Antitumor activity; *s*-tetrazine; X-ray analysis.
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† All new compounds were characterized by IR, ^{1}H NMR, MS and elemental analysis.

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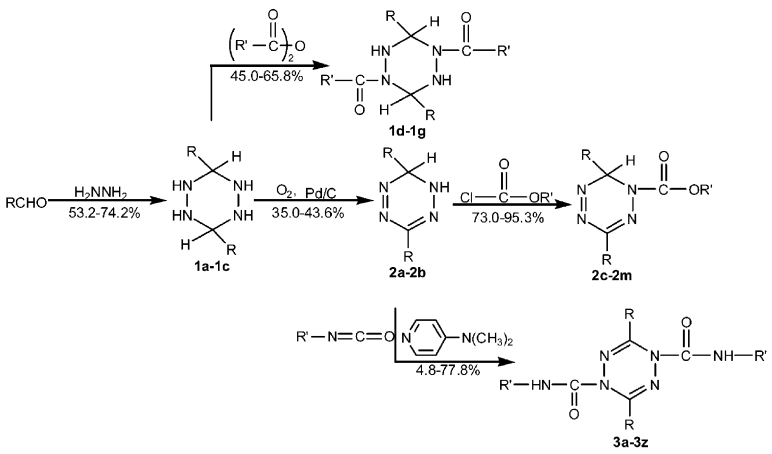
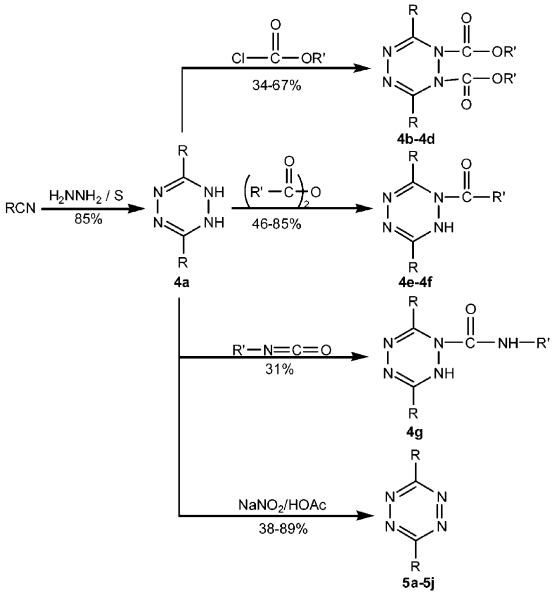


Scheme 1.



Scheme 2.

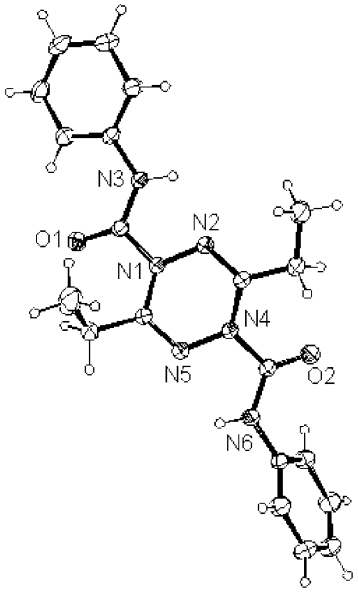


Fig.2. X-ray structure of **3d**.

effective to P-388. Among 1,4-dihydro-*s*-tetrazine (**3a**–**z**), there are 9 compounds which have strong effective to A-549 cell and 6 compounds having strong effective to P-388 cell. Especially, compounds **3i**, **3q**, **3t**, **3w**, **3z** have strong effective to both P-388 and A-549 cell. In addition, the substitutes on phenyl have much more effect on the activity. Electrondonor substitutes are of benefit to the inhibition ratio, and the compounds with *meta* electrondonor substitute have more strong effective than that compounds with the same substitute but at *ortho* or *para* position.

For more accurately to examine antitumor activity, the **3q** was selected to test the IC_{50}. The IC_{50} of **3q** for P-388, Bel-7402, MCF-7 and A-549 are 0.6 μM, 0.6 μM, 0.5 μM and 0.7 μM, respectively. The results were summarized in Table 3. So the *s*-tetrazine especially 1,4-dihydro-*s*-tetrazine is a kind of compound which may have potential antitumor activities. It is a good lead compound that warrants further investigation.

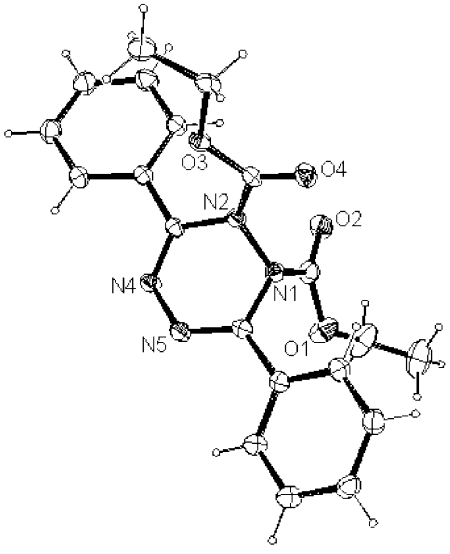


Fig.3. X-ray structure of **4c**.

Table 1. Physical data and elemental analysis

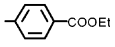
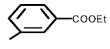
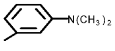
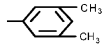
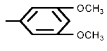
Compd	R	R′	mp (°C)	Yield (%)	Formula	Elem. Anal. (%) Cal./ Found		
						C	H	N
1a	Me		58–60[6c]	74.2				
1b	Et		127–131[6c]	53.2				
1c	*n*-Pr		129–130[6c]	56.9				
1d	Me	Me	200–216[6b]	45.0				
1e	Et	Me	184–193	65.8	$C_{10}H_{20}N_4O_2$	52.86/52.61	8.99/8.83	24.48/24.54
1f	*n*-Pr	Me	196–206	46.9	$C_{12}H_{24}N_4O_2$	56.22/56.26	9.44/9.52	21.86/21.83
1g	*n*-Bu	Me	188–190	63.4	$C_{14}H_{28}N_4O_2$	59.16/59.12	9.87/9.92	19.79/19.70
2a	Me		107–108[6a]	35.0				
2b	*n*-Pr		71–72[6c]	43.6				
2c	Me	Me	Liq.[6a]	92.9				
2d	Me	*n*-Pr	Liq.	86.9	$C_8H_{14}N_4O_2$	48.47/48.52	7.12/7.08	28.27/28.28
2e	Me	Ph	Liq.	95.3	$C_{11}H_{12}N_4O_2$	56.89/56.49	5.21/5.08	24.13/24.17
2f	Me	CH_2Ph	Liq.	86.2	$C_{12}H_{14}N_4O_2$	58.53/58.60	5.73/5.77	22.75/22.75
2g	Me	*n*-C_5H_{11}	Liq.	89.4	$C_{10}H_{18}N_4O_2$	53.08/52.95	8.02/8.00	24.76/24.69
2h	Me	*i*-C_5H_{11}	Liq.	83.2	$C_{10}H_{18}N_4O_2$	53.08/53.21	8.02/8.14	24.76/24.84
2i	*n*-Pr	Me	Liq.	83.2	$C_{10}H_{18}N_4O_2$	53.08/53.15	8.02/8.26	24.76/24.82
2j	*n*-Pr	Et	Liq.	81.7	$C_{11}H_{20}N_4O_2$	54.98/54.90	8.39/8.36	23.32/23.41
2k	*n*-Pr	Cyclohexyl	Liq.	85.0	$C_{15}H_{26}N_4O_2$	61.20/61.24	8.90/9.06	19.03/19.06
2l	*n*-Pr	*n*-C_5H_{11}	Liq.	92.9	$C_{14}H_{26}N_4O_2$	59.55/59.58	9.28/9.37	19.84/19.90
2m	*n*-Pr	*i*-C_5H_{11}	Liq.	73.0	$C_{14}H_{26}N_4O_2$	59.55/59.57	9.28/9.36	19.84/20.04
3a	*n*-Pr	Ph	128–130	47.7	$C_{22}H_{26}N_6O_2$	65.01/65.03	6.45/6.66	20.67/20.75
3b	*n*-Pr	*m*-CH_3Ph	91–93	34.5	$C_{24}H_{30}N_6O_2$	66.34/66.17	6.96/7.04	19.34/19.44
3c	*n*-Pr	*m*-ClPh	120–121	27.3	$C_{22}H_{24}Cl_2N_6O_2$	55.59/55.47	5.09/5.08	17.68/17.63
3d	Et	Ph	131–133	52.9	$C_{20}H_{22}N_6O_2$	63.48/63.40	5.86/5.88	22.21/22.38
3e	Et	*m*-CH_3Ph	90–91	24.9	$C_{22}H_{26}N_6O_2$	65.01/65.02	6.45/6.46	20.67/20.55
3f	Me	Cyclohexyl	184–186	5.8	$C_{18}H_{30}N_6O_2$	59.65/59.98	8.34/8.44	23.19/22.84
3g	Me	$PhCH_2$	154–156	20.7	$C_{20}H_{22}N_6O_2$	63.48/63.40	5.86/6.12	22.21/21.86
3h	Me	α-Naphthyl	223–224	4.8	$C_{26}H_{22}N_6O_2$	69.32/69.24	4.92/4.95	18.65/18.48
3i	Me	Ph	183–184	58.0	$C_{18}H_{18}N_6O_2$	61.70/61.76	5.18/5.08	23.99/23.97
3j	Me	*m*-CF_3Ph	204–206	32.4	$C_{20}H_{16}F_6N_6O_2$	49.39/49.73	3.32/3.39	17.28/17.59
3k	Me		216–217	77.8	$C_{24}H_{26}N_6O_6$	58.29/58.22	5.30/5.23	16.99/17.18
3l	Me	*m*-NO_2Ph	254–255	19.7	$C_{18}H_{16}N_8O_6$	49.09/48.85	3.66/3.75	25.44/25.18
3m	Me	*o*-ClPh	199–201	47.2	$C_{18}H_{16}Cl_2N_6O_2$	51.56/51.60	3.85/3.85	20.04/20.25
3n	Me	*m*-ClPh	210–211	64.7	$C_{18}H_{16}Cl_2N_6O_2$	51.56/51.75	3.85/4.08	20.04/20.09
3o	Me	*p*-ClPh	231–232	29.8	$C_{18}H_{16}Cl_2N_6O_2$	51.56/51.57	3.85/3.99	20.04/20.39
3p	Me	*o*-CH_3Ph	185–187	24.7	$C_{20}H_{22}N_6O_2$	63.48/63.71	5.86/5.93	22.21/22.49
3q	Me	*m*-CH_3Ph	138–139	37.5	$C_{20}H_{22}N_6O_2$	63.48/63.72	5.86/6.09	22.21/22.57
3r	Me	*p*-CH_3Ph	201–202	19.4	$C_{20}H_{22}N_6O_2$	63.48/63.62	5.86/5.63	22.21/22.46
3s	Me	*o*-CH_3OPh	198–199	7.8	$C_{20}H_{22}N_6O_4$	58.53/58.24	5.40/5.04	20.48/20.67
3t	Me	*m*-CH_3OPh	136–137	37.4	$C_{20}H_{22}N_6O_4$	58.53/58.55	5.40/5.34	20.48/20.67
3u	Me	*p*-CH_3OPh	180–182	28.7	$C_{20}H_{22}N_6O_4$	58.53/58.75	5.40/5.57	20.48/20.65
3v	Me		172–174	37.4	$C_{24}H_{26}N_6O_6$	58.29/58.45	5.30/5.45	16.99/17.13
3w	Me		170–172	20.0	$C_{22}H_{28}N_8O_2$	60.53/60.44	6.46/6.50	25.67/25.83
3x	Me		235–237	44.2	$C_{18}H_{14}Cl_4N_6O_2$	44.29/44.31	2.89/2.92	17.22/17.29
3y	Me		236–237	30.8	$C_{22}H_{26}N_6O_2$	65.01/65.29	6.45/6.53	20.67/20.54
3z	Me		230–231	52.5	$C_{22}H_{26}N_6O_6$	56.16/55.92	5.57/5.64	17.86/17.75
4a	Ph	H	194–196[6e]	85				
4b	Ph	Me	173–174	67	$C_{18}H_{16}N_4O_4$	61.36/61.34	4.58/4.56	15.90/15.94
4c	Ph	Et	121–122	34	$C_{20}H_{20}N_4O_4$	63.15/63.17	5.30/5.29	14.73/14.93
4d	Ph	*n*-Pr	111–112	64	$C_{22}H_{24}N_4O_4$	64.69/64.65	5.92/6.04	13.72/13.81
4e	Ph	Me	164–165	85	$C_{16}H_{14}N_4O$	69.05/69.14	5.07/5.03	20.13/20.22
4f	Ph	*i*-Pr	195–196	46	$C_{18}H_{18}N_4O$	70.57/70.76	5.92/6.00	18.29/18.10
4g	Ph	*o*-CH_3OPh	210–211	31	$C_{22}H_{19}N_5O_2$	68.56/68.66	4.97/4.86	18.17/18.02
5a	Pyrazinyl		200(d)	39	$C_{10}H_6N_8$	50.41/50.64	2.54/2.15	47.04/47.21
5b	Ph		194–196[6f]	83				
5c	*p*-CH_3Ph		220–231[6f]	38				
5d			179–180	47	$C_{14}H_{12}N_6$	63.61/63.79	4.59/4.82	31.40/31.80
5e	*m*-ClPh		208–210[6f]	44				
5f	*p*-ClPh		180–190[6f]	87				
5g	*o*-ClPh		178–180[6f]	52				
5h	$PhCH_2$		63–65[6f]	50				
5i	*p*-$ClPhCH_2$		131–132	57	$C_{16}H_{12}Cl_2N_4$	58.02/58.34	3.65/3.61	16.91/16.73
5j	*p*-CF_3Ph		250(d) [6f]	89				

Table 2. Inhibition of in vitro tumor cell growth by tetrazine derivatives

Compd	Rate of inhibition of P388, C (tetrazine)/(mol.L^{-1})					Rate of inhibition of A549, C (tetrazine)/(mol.L^{-1})				
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1d	14.5	0.1	4.3	0.0	0.0	68.5	6.0	3.6	7.4	3.2
1e	38.9	0.0	0.0	0.0	0.0	47.5	9.7	10.1	2.9	0.0
1f	38.5	5.5	3.2	3.1	0.0	59.8	8.6	4.0	1.2	0.0
1g	37.9	0.0	0.0	0.0	0.0	47.6	13.1	6.4	4.8	0.0
2a	100	19.7	9.2	15.9	0.0	67.7	4.8	0.0	5.5	0.0
2b	42.2	0.0	0.0	0.0	0.0	61.7	0.5	0.0	0.0	0.0
2c	60.5	7.8	0.0	11.3	5.7	75.9	19.6	0.0	0.0	0.0
2d	34.8	13.5	9.0	8.7	5.2	16.7	11.0	0.0	0.0	0.0
2e	85.3	27.9	13.6	1.2	0.0	32.1	0.0	0.0	0.0	0.0
2f	24.1	3.0	2.0	2.3	1.0	0.0	0.0	0.0	0.0	0.0
2g	42.6	19.8	7.3	0.0	0.0	22.8	4.6	0.0	1.1	7.6
2h	28.8	17.8	13.6	0.8	0.0	0.0	0.0	0.0	0.0	0.0
2i	90.4	17.3	4.5	1.7	3.3	59.6	0.0	0.0	0.0	0.0
2j	35.4	15.0	2.2	5.0	3.6	12.5	0.0	0.0	0.0	0.0
2k	44.0	33.6	38.3	44.8	4.5	25.8	5.2	23.5	18.1	1.0
2l	32.8	7.7	5.6	0.0	0.0	0.0	0.7	2.3	0.0	2.5
2m	83.5	14.3	6.9	7.3	9.9	89.4	0.0	0.0	0.0	0.0
3a	85.4	61.5	22.8	4.4	4.1	56.6	78.3	48.3	6.1	2.5
3b	81.6	63.9	0.0	4.8	0.0	93.3	84.2	74.1	4.0	0.0
3c	84.4	58.7	0.0	0.0	0.0	91.3	88.4	59.6	10.8	0.0
3e	91.6	63.5	0.0	0.0	0.0	90.4	84.1	81.1	16.7	1.6
3f	72.6	0.0	0.0	0.0	0.0	58.9	3.5	0.0	0.0	0.0
3g	92.8	57.5	50.3	22.0	19.6	64.4	82.3	81.8	32.0	14.1
3h	60.5	6.3	0.0	0.0	0.0	56.6	16.9	9.1	10.0	4.4
3i	89.1	90.6	90.6	87.5	81.3	76.5	77.9	79.4	60.3	50.0
3j	73.0	28.6	9.5	0.0	0.0	39.3	10.7	8.9	10.7	16.1
3k	63.5	25.4	1.6	0.0	0.0	27.3	28.8	0.0	0.0	0.0
3l	69.8	58.7	38.1	20.6	22.2	0.9	0.0	0.0	0.0	0.0
3m	90.5	41.3	1.6	0.0	0.0	28.8	0.0	0.0	0.0	0.0
3n	85.9	29.7	6.3	0.0	0.0	21.2	7.6	0.0	0.0	0.0
3o	50.0	14.8	1.9	7.4	0.0	53.4	14.8	1.9	7.4	0.0
3p	82.8	18.8	1.6	0.0	0.0	80.0	0.0	0.0	0.0	0.0
3q	96.8	96.8	98.4	96.8	96.8	84.8	83.3	80.3	81.8	80.3
3r	73.4	4.7	3.1	3.1	0.0	24.6	9.2	6.2	7.7	12.3
3s	59.4	17.2	1.6	0.0	0.0	80.0	0.0	0.0	0.0	0.0
3t	95.3	93.8	93.8	95.2	92.2	87.7	86.2	84.6	89.2	86.2
3u	73.0	36.5	14.3	6.3	0.0	24.2	0.9	0.0	0.0	0.0
3v	46.3	0.0	0.0	0.0	0.0	67.0	12.5	6.8	5.7	1.1
3w	95.4	84.3	89.8	88.9	90.7	93.2	83.0	80.7	73.9	69.3
3x	30.6	5.9	1.9	0.0	0.0	42.0	0.0	0.0	0.0	0.0
3y	90.7	91.7	31.5	0.0	0.0	86.4	73.9	37.5	0.0	0.0
3z	94.4	88.8	88.9	90.7	73.1	87.5	79.3	77.3	75.0	26.1
4a	55.8	67.2	5.4	10.2	0.0	87.6	69.0	0.0	0.0	0.0
4b	50.5	14.6	11.6	5.2	3.3	35.5	0.0	0.0	0.0	0.0
4c	93.9	35.6	7.0	4.9	2.8	95.3	10.8	0.0	0.0	0.0
4d	80.9	54.8	4.5	4.3	2.5	23.4	29.8	0.5	0.0	0.0
4e	62.7	10.9	6.6	5.5	9.2	85.1	0.0	0.0	0.0	0.0
4f	82.5	61.1	52.0	24.9	46.9	83.8	46.6	38.7	29.6	29.5
4g	64.5	25.2	7.4	0.8	0.0	90.9	72.6	4.7	0.0	0.0
5a	69.9	0.0	0.0	0.0	0.0	60.6	3.9	0.0	0.0	0.0
5b	15.5	0.0	0.0	0.0	0.0	60.4	9.5	20.3	27.1	29.7
5c	56.4	8.3	4.4	2.4	4.5	90.3	17.0	0.0	0.0	0.0
5d	2.6	3.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5e	40.3	26.5	33.2	13.5	4.3	0.0	0.0	0.0	0.0	0.0
5f	52.3	35.1	29.0	19.4	16.1	0.0	0.0	0.0	0.0	0.0
5g	8.2	2.8	5.6	14.6	12.6	0.0	0.0	0.0	0.0	0.0
5h	51.0	15.6	18.5	4.6	0.0	66.8	61.7	4.3	0.0	0.0
5i	58.4	29.1	9.3	8.9	0.0	65.8	57.1	13.1	1.1	8.7
5j	51.5	44.4	33.0	19.5	9.7	40.2	2.5	0.0	0.0	0.0

Table 3. Determine the IC_{50} of compound **3q**

Cancer cell	Rate of inhibition (%)																IC_{50} (μM)
	Concentration (μM)																
	0	5.0	3.125	2.5	1.563	1.25	1.0	0.781	0.625	0.5	0.399	0.313	0.25	0.195	0.125	0.0625	
P-388	—	—	97.3	—	91.1	—	—	83.1	—	—	55.4	—	—	2.3	—	—	0.6
Bel-7402	—	87.8	—	89.0	—	77.8	—	—	54.2	—	—	27.4	—	—	—	—	0.6
MCF-7	—	—	—	—	—	—	71.2	—	—	64.3	—	—	35.7	—	1.1	0	0.5
A-549	—	—	—	—	—	—	93.6	—	—	36.6	—	—	0	—	0	0	0.7

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8. Synthesis of **3d**: A mixture of 3,6-diethyl-1,6-dihydro-*s*-tetrazine (1.40 g, 10 mmol), 4-dimethylaminopyridine (0.50 g, 4 mmol), phenyl isocyanate (2.66 g, 20 mmol) and chloroform (50 cm^3) was heated at reflux for 72 h. After removing the solvent, *n*-hexane was added to the residue of the mixture, and then cooled. The resulting precipitate was filtered, and recrystallized from alcohol to give **3d** (2.00 g, 52.9%) as colorless crystal (Found: C, 63.40; H, 5.88; N, 22.38. Calc. for $C_{20}H_{22}N_6O_2$: C, 63.48; H, 5.86; N, 22.21%); Mp 131∼133 °C; ν_{max}/cm^{-1} 3340 (NH), 3040 (Ph), 2963, 2928 (CH_3), 1698, 1593, 1505 (Ph), 1443, 1317 (C=N), 1222, 973, 756 and 735; δ_H (400 MHz; $CDCl_3$; Me_4Si) 9.25 (2H, s, NH), 7.10–7.60 (10H, m, Ph), 2.89 (4H, q, CH_2) and 1.15 (6H, t, CH_3); *m*/*z* 378 (M^+, 13%), 141(9), 140(100), 119(12), 91(12), 77(17), 65(9), 56(49). Compounds of **3a–z** were prepared as described above for **3d**.
9. Crystal data of **3d**. $C_{20}H_{22}N_6O_2$, $M=378.44$, Orthorhombic, $a=16.287(2)$, $b=11.347(4)$, $c=20.975(4)$ Å, $U=3876.4(16)$ $Å^3$, $T=293(2)$ K, space group *Pbca* (no. 61), $Z=8$, $D_c=1.297$ g cm^{-3}, μ (Mo-K_α) = 0.088 mm^{-1}, 3856 reflections measured, 3486 unique ($R_{int}=0.0284$) which were used in all calculations. Fine $R_1=0.034$, $wR(F^2)=0.1035$ (all data). CCDC reference number 220758.
10. Crystal data of **4c**. $C_{20}H_{20}N_4O_4$, $M=380.40$, triclinic, $a=8.915(2)$, $b=10.444(2)$, $c=11.509(3)$ Å, $\alpha=103.268(3)$, $\beta=102.844(3)$, $\gamma=100.765(3)$ Å, $U=984.4(4)$ $Å^3$, $T=293(2)$ K, space group P_{-1} (no. 2), $Z=2$, $D_c=1.283$ g cm^{-3}, μ(Mo-K_α) = 0.092 mm^{-1}, 4947 reflections measured, 4179 unique ($R_{int}=0.0188$) which were used in all calculations. Fine $R_1=0.059$, $wR(F^2)=0.1969$. CCDC reference number 220757.