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Original article

Biological evaluation of polyhalo 1,3-diazaheterocycle fused isoquinolin-1(2*H*)-imine derivatives

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ABSTRACT

A series of polyhalo 1,3-diazaheterocycle fused isoquinolin-1(2*H*)-imines were evaluated *in vitro* against human tumour cell lines including A431, K562, HL60, HepG2 and Skov-3. As a result, some of the target compounds such as **5b**, **5c**, **5i**, **5o**, **6c**, **6h** and **7f** showed stronger cytotoxicity against K562, H562 and Skov-3 cells in comparison with cisplatin, and the others displayed moderate cytotoxicity to A431 and HepG2. Biological investigations using the representative compounds **5c**, **6c** and **6h** were also performed in mice bearing S_{180} and H_{22} tumours. The results indicated that these three compounds inhibit S_{180} and H_{22} growth. In addition, compounds **6c** and **6h** have very low acute toxicities. The preliminary analysis of structure–activity relationships is also discussed.

1. Introduction

Isoquinolin-1(2*H*)-imine derivatives exhibit various biological and medicinal activities, inhibiting acetylcholinesterase (AChE) and butyryl-cholinester-ase (BChE) [1], and having antimalarial [2], anti-inflammatory, anti-nociceptive [3], antitumour, antiviral, antibacterial and antifungal properties [4]. However, the efficient methods for the production of isoquinolin-1(2*H*)-imines are very rare [5,6]. Therefore, the development of effective methodologies for constructing molecular libraries of isoquinolinimine with great potential for drug discovery would be desirable.

Polyhalo isophthalonitriles, especially polyfluoro-isophthalonitrile, have been widely used as anti-cancer [7–9], antiinflammatory [10] and insecticidal [11] agents, and so on [12] in pharmaceuticals. Fluorine-containing compounds often show improved biological activity profiles being their superior metabolic stability and the lipophilicity of fluorine. In this context, F-containing compounds have been attracting considerable attention in the field of pharmaceutical chemistry [13].

Heterocyclic ketene aminals (HKAs) are versatile substrates for the synthesis of fused heterocyclic compounds with a wide variety of uses, including as anti-cancer agents [7], herbicides, pesticides, anti-anxiety agents [14,15], anti-leishmanial agents [16] and anti-bacterial drugs [17].

Our previous studies [7] have also shown that the polyhalo heterocyclic ketene aminals (polyhalo-HKAs), particularly trifluoro-HKAs, possess strong cytotoxicity against human carcinoma cells *in vitro*. In this regard, we envisage that the polyhalo isoquinolinimines starting from the easily available polyhalo isophthalonitriles and HKAs would also possess potential bioactivity.

In the present study, a series of isoquinolin-1(2*H*)-imines bearing multiple halogen atoms in the benzene ring and different types of substituted acyl groups at position-4 of the isoquinolin-1(2*H*)-imine ring were synthesized. The *in vitro* and *in vivo* anti-tumour activities of isoquinolin-1(2*H*)-imines and their acute toxicity were further evaluated, and a preliminary study on the structure–activity relationship was also carried out.

2. Results and discussion

2.1. Chemistry

The synthetic routes of compounds **5**–**7** are outlined in Scheme 1. A mixture composed of a 1:1.1 M ratio of HKAs **1**–**2** or *N*,*O*-acetals **3** to polyhalo isophthalonitrile **4** was treated under microwave irradiation (MW) in solvent-free conditions (120 °C for 12 min with a maximum power of 200 W). Then the mixture was directly mixed

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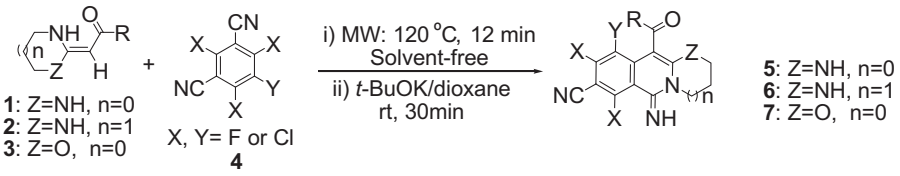


Scheme 1. Synthetic route of novel polyhalo 1,3-diazaheterocycle fused isoquinolin-1(2*H*)-imine derivatives **5**–**7**.

with 1.1 equiv. of *t*-BuOK at room temperature for 30 min. Isoquinolin-1(2*H*)-imines **5**–**7** were isolated in excellent yields of 79–96%.

The structures of the target compounds i.e. **5**–**7** were characterized by IR, ^{1}H NMR, ^{13}C NMR, ^{19}F NMR and the high resolution mass spectrometry (HRMS) [18]. Their physical properties are summarized in Table 2.

2.2. X-ray analysis of compound 5c

The three-dimensional structure of compound **5c** was further determined by X-ray crystallography (as shown in Fig. 1, the Cambridge crystallographic data centre (CCDC) 736001 [19]). The results showed that there are two kinds of intramolecular hydrogen bonds: the hydrogen bond between the carbonyl group and the secondary amine on the imidazole ring N3–H3···O1 (Table 1, entry 4), and the hydrogen bond N4–H4···F1 (Table 1, entry 1). In addition, intermolecular hydrogen bonds such as N(4)–H(4)···O(2) (Table 1, entry 2) and N3–H3···O3 (Table 1, entry 3) were also observed. A summary of hydrogen bonding geometry is given in Table 1.

2.3. In vitro evaluation of cytotoxicity

The cytotoxicity of individual compounds against human myeloid leukaemia cells (HL60 and K562), human epidermoid carcinoma cells (A431), human ovarian carcinoma cells (Skov-3) and human laryngeal carcinoma cells (Hep-2) was evaluated through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colourimetric assay [20,21], using cisplatin (DDP) as the positive control. The IC_{50} values are presented in Table 2 (IC_{50} value, defined as the concentration corresponding to 50% growth inhibition). The majority of compounds **5**–**7** displayed superior cytotoxicity compared to DDP against the HL60 and K562 cells. However, cytotoxicity against A431, Skov-3 and Hep-2 was similar to DDP in most cases. Typically, **5b** (IC_{50} 0.0008 μg/mL), **5c** (IC_{50} 0.0005 μg/mL) and **7f** (IC_{50} 0.0002 μg/mL) were more than 1250 times more active than DDP (IC_{50} 1 μg/mL) against K562 cells. The activity of **5b** (IC_{50} 0.003 μg/mL), **5i** (IC_{50} 0.001 μg/mL) and **7f** (IC_{50} 0.008 μg/mL) was more than 62 times higher than that of DDP (IC_{50} 0.5 μg/mL) against HL60 cells. Compounds **5b** (IC_{50} 0.05 μg/mL), **5c** (IC_{50} 0.04 μg/mL) and **5o** (IC_{50} 0.02 μg/mL) were more than 78 times more active than DDP (IC_{50} 3.9 μg/mL) against Skov-3 cells.

The different ring-sizes of the isoquinolin-1(2*H*)-imines ($n = 0, 1$) appeared to influence bioactivity. For instance, the bioactivity of five-membered isoquinolin-1(2*H*)-imines ($n = 0$) was extremely different from that of the six-membered isoquinolin-1(2*H*)-imines ($n = 1$) under the experimental conditions (Table 2, entries 5–7 vs. 17–19; 8–10 vs. 23–25; 11–13 vs. 26–28). These results also suggest that the isostere of N and O (Z=NH, O) does not play an important role in the modulation of cytotoxic activity to different tumour cell lines. Consequently, imidazo-[1,2-*b*]isoquinolin-imines **5a**–**5o** and oxazo-[1,2-*b*]isoquinolin-imines **7a**–**7f** showed slight different activity (Table 2, entries 5–10 vs. 29–34).

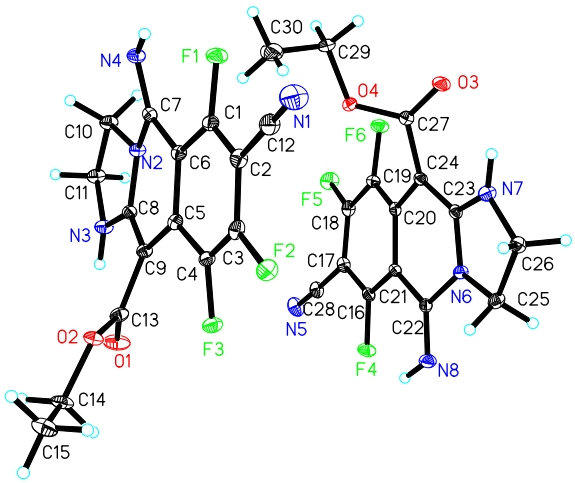


Fig. 1. X-ray crystal structure of **5c**.

2.4. Structure–activity relationship (SAR)

The cytotoxicity data suggest that the fluorine atom in the title compounds plays an important role in promoting biological activity. Moreover, cytotoxic activity increased with an increase in the number of fluorine atoms (Table 2, entry 4 vs. 3 vs. 2). In particular, **5a**, **5b**, and **5c** showed a different IC_{50} range against the tumour cell lines Hep-2 (IC_{50} 38.2–0.4 μg/mL), Skov-3 (IC_{50} 50.1–0.04 μg/mL) and K562 (IC_{50} 1.6–0.0005 μg/mL). This is presumably because the fluorine atoms decrease the Gibbs Energy (Table 2, entries 3–4 vs. 2; 15–16 vs. 14; 3–4 vs. 5–34). The lower Gibbs Energy maybe contribute to facile formation of the intramolecular and intermolecular hydrogen bonds (Fig. 1 and Table 1). For example, **5m**, **6a** and **6d** maybe cannot to form intramolecular hydrogen bonds N–H···Cl and showed little cytotoxicity. In addition, this kind of intramolecular hydrogen bond i.e. N–H···F would make a significant positive contribution to cytotoxicity (Table 2, entries 3–4 vs. 2; 6–7 vs. 5; 9–10 vs. 8; 12–13 vs. 11; 15–16 vs. 14).

Lipophilicity is the most important aspect for drugs to pass through the blood–brain barrier. Lipophilicity is described by log *P* values. The predicted log *P* values of the isoquinolin-1(2*H*)-imine derivatives **5**–**7** were within the range of 1.03–4.84. We found that

Table 1
Hydrogen bonding geometry (Å, °).

No.	D–H···A	d(D–H)	d(H···A)	d(D···A)	<(DHA)
1	N(4)–H(4)···F(1)	0.87 (2)	2.19 (2)	2.820 (2)	129.3 (18)
2	N(4)–H(4)···O(2)^{i}	0.87 (2)	2.49 (2)	3.280 (2)	151.5 (18)
3	N(3)–H(3)···O(3)^{ii}	0.88 (2)	2.17 (2)	2.977 (2)	152.0 (19)
4	N(3)–H(3)···O(1)	0.88 (2)	2.22 (2)	2.787 (2)	121.8 (17)
5	N(8)–H(8)···F(4)	0.88 (2)	2.13 (2)	2.807 (2)	133.5 (18)
6	N(7)–H(7)···O(1)^{iii}	0.89 (2)	2.06 (2)	2.885 (2)	153.0 (20)
7	N(7)–H(7)···O(3)	0.89 (2)	2.28 (2)	2.808 (2)	118.0 (18)

Symmetry codes: (i) $-x + 1, -y, -z + 1$; (ii) $x - 1, y, z$; (iii) $x + 1, y, z$.

Table 2
Isoquinolin-1(2*H*)-imines and their physicochemical properties and biological activities[a] (IC_{50}, μg/mL[b]).

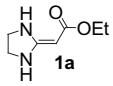
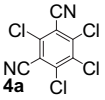
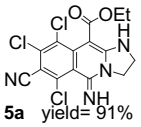
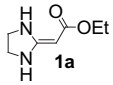
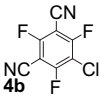
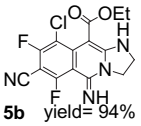
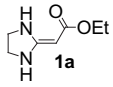
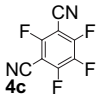
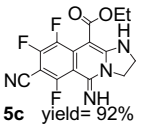
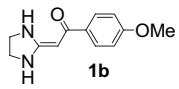
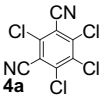
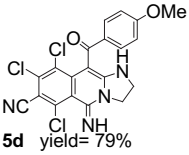
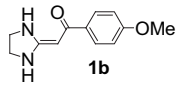
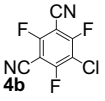
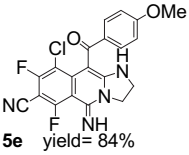
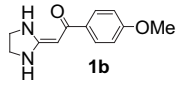
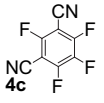
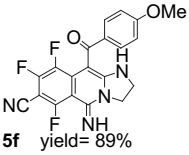
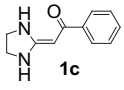
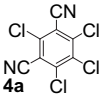
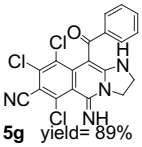
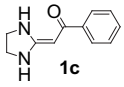
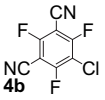
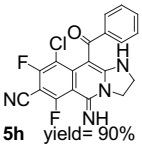
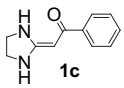
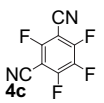
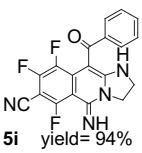
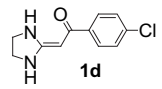
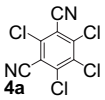
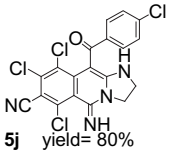
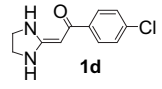
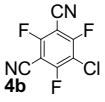
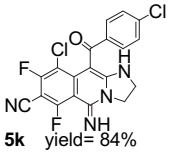
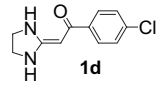
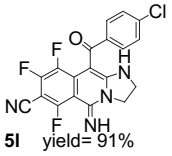
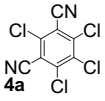
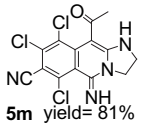
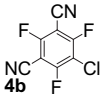
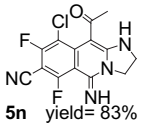
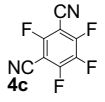
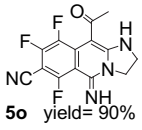
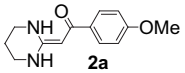
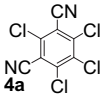
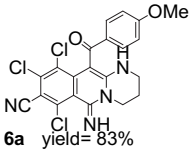
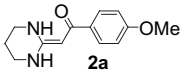
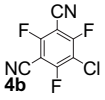
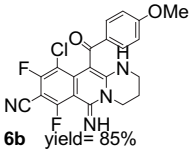
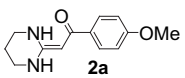
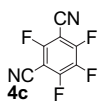
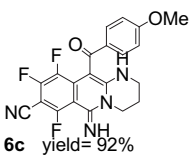
No.	1–3	4	Compound	Gibbs Energy[c] [kJ/mol]	log *P*	Anti-cancer activity IC_{50} (μg/mL)				
						A431	HL60	HepG2	Skov-3	K562
1			DDP			1.1	0.5	0.06	3.9	1
2	 **1a**	 **4a**	 **5a** yield= 91%	450.2	3.44	40.6	1.7	38.2	50.1	1.6
3	 **1a**	 **4b**	 **5b** yield= 94%	84.44	2.68	2.2	0.003	1.5	0.05	0.0008
4	 **1a**	 **4c**	 **5c** yield= 92%	−98.44	2.04	3.4	0.02	0.4	0.04	0.0005
5	 **1b**	 **4a**	 **5d** yield= 79%	671.53	4.02	2.1	0.3	11.3	9.2	0.7
6	 **1b**	 **4b**	 **5e** yield= 84%	305.77	3.26	1.9	0.2	1.2	4.8	0.3
7	 **1b**	 **4c**	 **5f** yield= 89%	122.89	2.62	7.5	0.5	0.9	2.4	0.4
8	 **1c**	 **4a**	 **5g** yield= 89%	777.74	4.03	3.4	0.8	4.4	19.7	0.7
9	 **1c**	 **4b**	 **5h** yield= 90%	411.98	3.27	4.8	0.1	0.3	5.8	0.4
10	 **1c**	 **4c**	 **5i** yield= 94%	229.1	2.63	0.8	0.001	1.2	0.2	0.08

Table 2 (*continued*).

No.	1–3	4	Compound	Gibbs Energy[c] [kJ/mol]	log *P*	Anti-cancer activity IC_{50} (μg/mL)				
						A431	HL60	HepG2	Skov-3	K562
11	 **1d**	 **4a**	 **5j** yield= 80%	756.18	4.76	20.3	2.6	37.6	31.4	2.4
12	 **1d**	 **4b**	 **5k** yield= 84%	390.42	4.00	5	0.3	12.4	34.9	1.4
13	 **1d**	 **4c**	 **5l** yield= 91%	207.54	3.36	4.9	0.5	17.7	34.5	1
14	 **1e**	 **4a**	 **5m** yield= 81%	623.23	2.43	308	4.7	503	78.4	3.6
15	 **1e**	 **4b**	 **5n** yield= 83%	257.47	1.67	20.4	0.9	6.4	6.2	0.4
16	 **1e**	 **4c**	 **5o** yield= 90%	74.59	1.03	4.4	0.02	3.1	0.02	0.1
17	 **2a**	 **4a**	 **6a** yield= 83%	667.85	4.10	56.9	4.4	400	67.2	1.4
18	 **2a**	 **4b**	 **6b** yield= 85%	302.09	3.34	2	0.5	0.8	26.8	0.07
19	 **2a**	 **4c**	 **6c** yield= 92%	119.21	2.70	1.1	0.02	0.8	2.6	0.01

(*continued on next page*)

Table 2 (*continued*).

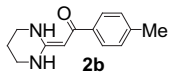
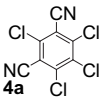
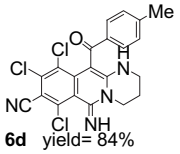
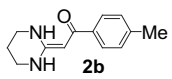
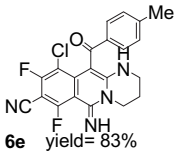
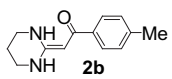
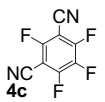
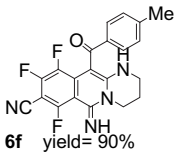
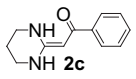
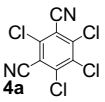
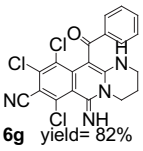
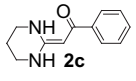
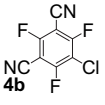
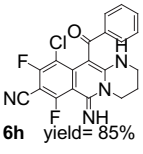
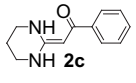
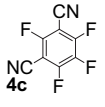
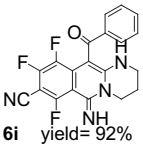
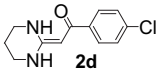
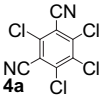
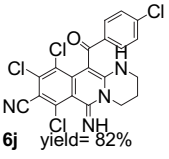
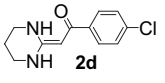
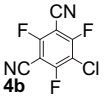
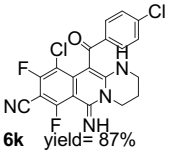
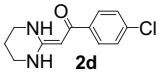
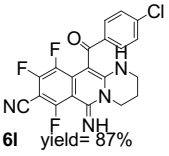
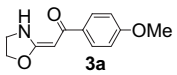
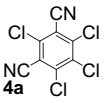
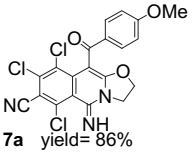
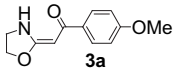
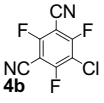
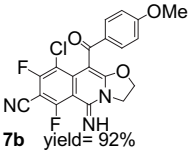
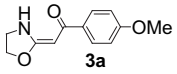
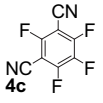
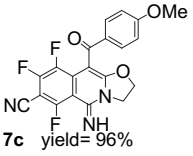
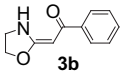
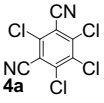
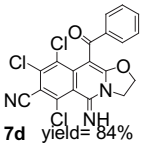
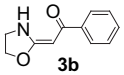
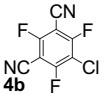
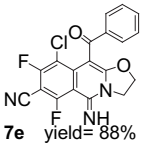
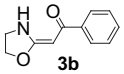
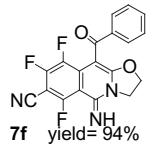
No.	1–3	4	Compound	Gibbs Energy[c] [kJ/mol]	log *P*	Anti-cancer activity IC_{50} (μg/mL)				
						A431	HL60	HepG2	Skov-3	K562
20	 **2b**	 **4a**	 **6d** yield= 84%	772.85	4.61	>1000	58.2	277	826	9.3
21	 **2b**	 **4b**	 **6e** yield= 83%	407.09	3.85	27.5	14.6	24.6	76.4	2.3
22	 **2b**	 **4c**	 **6f** yield= 90%	224.21	3.21	2.1	4.7	2.4	33.9	0.7
23	 **2c**	 **4a**	 **6g** yield= 82%	774.06	4.11	10.1	10.7	9.3	51.2	1.2
24	 **2c**	 **4b**	 **6h** yield= 85%	408.3	3.35	0.5	0.01	0.3	1.2	0.03
25	 **2c**	 **4c**	 **6i** yield= 92%	225.42	2.71	2.4	0.3	1.1	16.4	0.02
26	 **2d**	 **4a**	 **6j** yield= 82%	752.5	4.84	8	4.4	30	52.2	1.2
27	 **2d**	 **4b**	 **6k** yield= 87%	386.74	4.08	2.8	0.2	2.1	17.1	0.3
28	 **2d**	 **4c**	 **6l** yield= 87%	203.86	3.44	4.3	4.7	8.4	15.1	0.5

Table 2 (*continued*).

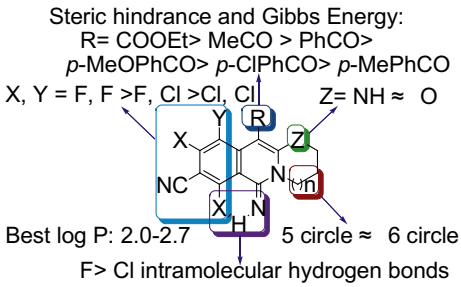
No.	1–3	4	Compound	Gibbs Energy[c] [kJ/mol]	log *P*	Anti-cancer activity IC_{50} (μg/mL)				
						A431	HL60	HepG2	Skov-3	K562
29	 **3a**	 **4a**	 **7a** yield= 86%	375.53	3.39	11	24.2	2.7	32.2	0.8
30	 **3a**	 **4b**	 **7b** yield= 92%	131.94	3.21	2.1	1	2	23.8	0.5
31	 **3a**	 **4c**	 **7c** yield= 96%	−50.94	2.57	1.5	0.1	0.5	0.2	1.8
32	 **3b**	 **4a**	 **7d** yield= 84%	603.91	3.97	14.5	6	0.3	17.4	2.2
33	 **3b**	 **4b**	 **7e** yield= 88%	238.15	3.21	2.3	1.5	3.6	29.2	1.7
34	 **3b**	 **4c**	 **7f** yield= 94%	55.27	2.57	0.3	0.008	0.1	0.2	0.0002

[a] Cytotoxicity as IC_{50} for each cell line, refers to the concentration of compound which reduced by 50% the optical density of treated cells with respect to untreated cells using the MTT assay.
[b] Data represent the mean values of three independent determinations.
[c] Gibbs Energy and log *P* values of the compounds were calculated by ChemDraw Ultra 11.0.

the chlorine atom had a stronger impact on the lipophilicity than the fluorine atom. For example, the lipophilicity of **5a** (log *P*: 3.44) was higher than that of **5b** (log *P*: 2.68) and **5c** (log *P*: 2.04). As a result, cytotoxicity decreased as lipophilicity increasing. Indeed, isoquinolin-1(2*H*)-imines with log *P* values of 2.0–2.7, such as **5b**, **5c**, **5i**, **6c**, and **7f**, showed better activity, and in particular, those compounds containing three fluorine atoms showed excellent cytotoxic activity ($IC_{50} < 3.4$ μg/mL) against the five human cancer cell lines. On the contrary, **6a** and **6d** (log *P*: 4.10–4.61) with three chlorine atoms each displayed weaker cytotoxic activity ($IC_{50} > 1.4$ μg/mL).

Isoquinolin-1(2*H*)-imines **5**–**7** bearing the same substituents exhibited different levels of cytotoxic activity, probably due to differences in steric hindrance and Gibbs Energy. Consequently, the cytotoxicity of various groups at position-4 was ranked as follows: ester > acetyl > benzoyl > *p*-methoxy-benzoyl > *p*-chlorine-benzoyl > *p*-methyl-benzoyl (Scheme 2).

In short, the number of fluorine atoms and intramolecular hydrogen bonds between the imine and the group at position-4 has a positive influence on the cytotoxic properties of isoquinolin-1(2*H*)-imine. For example, the compounds with two or three fluorine atoms and an ester or benzoyl group at position-4 (**5b**, **5c**, **5i** and **7f**) showed better cytotoxicity. In contrast, the compounds containing three chlorine atoms and an acetyl, *p*-methoxy-benzoyl or *p*-methyl-benzoyl group (**5m**, **6a** and **6d**) showed lower cytotoxicity against human cancer cells. Interestingly, compound **5c**



Scheme 2. Structure–activity relationship of isoquinolin-1(2*H*)-imines.

possessed a broad spectrum of cytotoxicity against the five human tumour cell lines (IC_{50} 0.0005–3.4 μg/mL), being 25–2000-fold more active than DDP except against the A431 and HepG2 cell lines. As a consequence, the compounds containing a framework with three fluorine atoms on the ring of isophthalonitrile and an electron withdrawing group (COOEt) at position-4 of isoquinolin-imine would be promising leads for further structural modifications according to the valuable information originating from the detailed SARs.

2.5. In vivo anti-tumour activity

To evaluate the *in vivo* anti-tumour activities of the representative compounds **5c**, **6c** and **6h**, their effects on the tumour weights of S_{180} and H_{22} mice were determined.

2.5.1. The effect of *5c*, *6c* and *6h* on S_{180} growth

Following daily oral administration of compound **5c** at dosages of 10, 30 and 90 mg/kg for 10 days, S_{180} growth inhibition rates were 39.25%, 54.17% and 26.62%, respectively. For compounds **6c** and **6h** at dosages of 20, 60 and 180 mg/kg, the S_{180} growth inhibition rates were 35.85%, 38.04%, 19.13% and 42.76%, 41.09%, 52.79%, respectively (Table 3). These results indicate that compounds **5c**, **6c** and **6h** have an inhibitory effect on S_{180} growth without an obvious dose-dependent relationship.

2.5.2. The effect of *5c*, *6c* and *6h* on H_{22} growth

Similarly, following daily oral administration of compound **5c** at dosages of 10, 30 and 90 mg/kg for 10 days, H_{22} growth inhibition rates were 11.44%, 27.52% and 31.90%, respectively. For compound **6c** at dosages of 35, 70 and 140 mg/kg, the inhibitory rates were 34.74%, 45.61% and 29.29%, respectively (Table 4). For compound **6h** at dosages of 20, 80 and 240 mg/kg, the inhibitory rates were 12.10%, 28.19% and 0%, respectively (Table 4). These results also show that compounds **5c**, **6c** and **6h** have an inhibitory effect on H_{22} growth, but with lower activity in comparison with that against S_{180}, and that there is no obvious dose-dependent relationship.

Table 3
The effect of **5c**, **6c** and **6h** on S_{180} growth.

Compounds	Dose (mg/kg)	Tumour weight (g) ($X \pm SD$)	Inhibitory rate %
Solvent (CMC-Na)	Equal volume	2.89 ± 0.62	–
Positive (cyclophosphamide)	20	0.53 ± 0.52	81.72
5c	10	1.76 ± 0.54	39.25
5c	30	1.33 ± 1.25	54.17
5c	90	2.12 ± 0.60	26.62
6c	20	1.86 ± 1.46	35.85
6c	60	1.79 ± 1.65	38.04
6c	180	2.34 ± 1.38	19.13
6h	20	1.66 ± 1.29	42.76
6h	60	1.70 ± 1.09	41.09
6h	180	1.37 ± 1.09	52.79

Table 4
The effect of **5c**, **6c** and **6h** on H_{22} growth.

Compounds	Dose (mg/kg)	Tumour weight (g) ($X \pm SD$)	Inhibitory rate %
Solvent (CMC-Na)	Equal volume	1.34 ± 0.33	–
Positive (cyclophosphamide)	20	0.59 ± 0.30	56.24
5c	10	1.19 ± 0.43	11.44
5c	30	0.97 ± 0.68	27.52
5c	90	0.91 ± 0.42	31.90
6c	35	0.87 ± 0.45	34.74
6c	70	0.73 ± 0.35	45.61
6c	140	0.95 ± 0.47	29.29
6h	20	1.18 ± 0.52	12.10
6h	80	0.96 ± 0.39	28.19
6h	240	1.34 ± 0.69	0.00

2.6. Preliminary acute toxicity test

Oral administration of compounds **6c** and **6h** at the dosages of 5000 mg/kg did not induce death in the mice tested, but compound **5c** at the dosage of 4500 mg/kg did result in death (4/6). This indicates that **6c** and **6h** would be lower acute toxicity than that of **5c**.

3. Conclusions

In summary, a series of polyhalo 1,3-diazaheterocycle fused isoquinolin-1(2*H*)-imines were evaluated for their cytotoxicity against A431, K562, HL60, HepG2 and Skov-3 carcinoma cells *in vitro*. Some of the target compounds such as **5b**, **5c**, **5i**, **5o**, **6c**, **6h** and **7f** showed stronger cytotoxicity against K562, H562 and Skov-3 cells than DDP, and the others displayed moderate cytotoxicity to the tumour cells A431 and HepG2. The preliminary structure–activity relationship analysis revealed that the presence of fluorine atoms and intramolecular hydrogen bonds between imine and fluorine could play an important role in enhancing biological activity. The representative compounds **5c**, **6c** and **6h** showed good anti-tumour activity *in vivo* the S_{180} and H_{22} mice models, but the potency depended on tumour type. In this regard, S_{180} was more sensitive than H_{22}. In addition, compounds **6c** and **6h** exhibited better levels of safety. These results direct the development of effective drugs for pharmaceutical application.

4. Experimental section

4.1. Chemistry

4.1.1. General methods

All compounds were fully characterized by spectroscopic techniques. The NMR spectra were recorded on a Bruker DRX500 spectrometer (^1H: 500 MHz, ^{13}C: 125 MHz, ^{19}F: 470 MHz) with tetramethylsilane (TMS) as the internal standard (δ 0.0 ppm), chemical shifts (δ) are expressed in ppm, and J values are given in Hz. Deuterated DMSO-d_6 was used as a solvent. IR spectra were recorded on a FT-IR Thermo Nicolet Avatar 360 using a KBr pellet. The reactions were monitored by thin layer chromatography (TLC)

using silica gel GF_{254}. The melting points were determined on an XT-4A melting point apparatus and are uncorrected. HRMS was performed on an Agilent LC-MSD TOF instrument.

All chemicals and solvents were used as received without further purification unless otherwise stated. Column chromatography was performed on silica gel (200–300 mesh).

The materials **1b**–**1e** and **2** were synthesized according to the literature [22]. Compounds **1a** and **3** were prepared based on published procedures [23]. **4a**–**4c** were purchased from Aldrich.

4.1.2. General procedure for the synthesis of polyhalo 1,3-diazaheterocyclic fused [1,2-b]iso-quinolin-1(2H)-imine **5**–**7**

A dry mortar was charged with HKAs **1**, **2**, **3** (1 mmol) and polyhalo isophthalonitrile **4** (1.1 mmol). The mixture was mixed at room temperature by vigorously grinding with a pestle for a few minutes (*ca*.1–2 min), then placed in a microwave tube and irradiated in a microwave reactor (Discover) with control of power and temperature by infrared detection at 120 °C for 12 min (maximum power 200 W). After cooling, the resulting mixture was transferred to a 50 mL flask, and dissolved in 25 mL 1,4-dioxane, before adding *t*-BuOK (1.5 mmol). While stirring at room temperature, the reaction was monitored by TLC. Upon completion, the resultant was poured into 60 mL of water and then filtered to obtain the crude products, which were purified by column chromatography (petrol:ethyl acetate = 1:3, *v*/*v*) on silica-gel to give the desired products **5**–**7**.

4.1.2.1. Ethyl 5-imino-6,8,9-trichloro-7-cyano-1,2,3,5-tetrahydroimidazo [1,2-b]isoquinoline-10-carboxylate (5a).

Yellow solid (0.351 g, 91%); Mp 234–236 °C; IR (KBr) (ν_{max}, cm^{-1}) 3360, 2223, 1670, 1304, 1158, 1037, 803, 641; ^1H NMR (500 MHz, DMSO-d_6) δ 9.30 (br, 1H, NH), 8.30 (br, 1H, NH), 4.16 (q, J = 7.1 Hz, 2H, OCH_2), 3.99 (t, J = 8.8 Hz, 2H, NCH_2), 3.76 (t, J = 8.7 Hz, 2H, NCH_2), 1.21 (t, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.2, 156.3, 151.6, 141.9, 137.1, 133.8, 125.8, 117.8, 114.3, 107.5, 80.5, 59.7, 45.2, 43.8, 14.1; HRMS (TOF ES$^-$): *m*/*z* calcd for $C_{15}H_{10}Cl_3N_4O_2^-$ [M^-], 382.9875; found, 382.9876.

4.1.2.2. 6-Imino-7,9,10-trichloro-11-(4-methoxybenz-oyl)-2,3,4,6-tetrahydro-1H-pyrimido[1,2b]-isoquinol-ine-8-carbonitrile (**6a**).

Yellow solid (0.383 g, 83%); Mp 204–206 °C; IR (KBr) (ν_{max}, cm^{-1}) 3436, 2226, 1596, 1261, 1162, 1029, 842, 604; ^1H NMR (500 MHz, DMSO-d_6) δ 10.14 (br, 1H, NH), 9.85 (br, 1H, NH), 7.41 (br, 2H, ArH), 6.83 (d, J = 7.4 Hz, 2H, ArH), 3.88 (br, 2H, NCH_2), 3.77 (s, 3H, OCH_3), 3.45 (br s, 2H, NCH_2), 2.08 (br s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 188.7, 162.1, 154.7, 153.5, 142.2, 137.0, 134.7, 133.5, 130.5, 125.9, 118.7, 114.8, 113.8, 107.5, 89.1, 55.7, 42.9, 38.0, 19.9; HRMS (TOF ES$^-$): *m*/*z* calcd for $C_{21}H_{14}Cl_3N_4O_2^-$ [M^-], 459.0188; found, 459.0185.

4.1.2.3. 6,8,9-Trichloro-5-imino-10-(4-methoxybenz-oyl)-3,5-dihydro-2H-oxazolo[3,2-b]isoquin-oline-7-carbonitrile (**7a**).

Yellow solid (0.386 g, 86%); Mp 287–289 °C; IR (KBr) (ν_{max}, cm^{-1}) 3428, 3261, 2237, 1618, 1440, 1251, 974, 780; ^1H NMR (500 MHz, DMSO-d_6) δ 10.29 (br, 1H, NH), 7.10 (d, J = 8.3 Hz, 2H, ArH), 6.79 (d, J = 8.4 Hz, 2H, ArH), 4.55 (t, J = 8.5 Hz, 2H, OCH_2), 3.89 (br s, 2H, NCH_2), 3.73 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 186.8, 167.7, 161.4, 148.9, 140.8, 138.7, 136.3, 133.8, 130.1, 117.9, 114.8, 114.7, 114.1, 113.7, 87.9, 69.3, 56.0, 44.6; HRMS (TOF ES$^-$): *m*/*z* calcd for $C_{20}H_{11}Cl_3N_3O_3^-$ [M^-], 445.9871; found, 445.9870.

4.1.3. X-ray crystallography for compound **5c**

Data collection: X-AREA; cell refinement: X-AREA; data reduction: X-RED32; program used to solve structure: SHELXS97; program used to refine structure: SHELXL97. CCDC 736001 contains the supplementary crystallographic data for compound **5c**. These data can be obtained free of charge from The Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/datarequest/cif.

4.2. In vitro cytotoxic activity [24]

Growth inhibition by the sample of tumour cells was measured by microculture tetrazolium (MTT) assay. Myeloid leukaemia (HL60 and K562), epidermoid carcinoma (A431), ovarian carcinoma (Skov-3), laryngeal carcinoma (Hep-2) cells were seeded into 96-well microculture plates, for the adherent cells, the cell were allowed culture 24 h for adhesion before drug addition, while suspended cells were seeded just before drug addition. The cell densities were selected based on the results of preliminary tests, in order to maintain the control cells in an exponential phase of growth during the period of the experiment and to obtain a linear relationship between the optical density and the number of viable cells. Each tumour cell line was exposed to sample at 0.01, 0.1, 1.0, 10 and 100 μg/mL concentrations for different periods (adherent cells 72 h, suspended cells 48 h) and each concentration was tested in triplicate. At the end of exposure, 20 μL of 5 g per litre MTT was added to each well and the plates were incubated for 4 h at 37 °C. Then triplex solution (10% SDS–5% isobutanol–0.012 M HCl) was added and the plates were incubated for 12–20 h at 37 °C. The optical density (OD) was read on a plate reader at 570 nm. Media and DMSO control wells, in which sample was absent, were included in all the experiments, in order to eliminate the influence of DMSO. The inhibitory rate of cell proliferation was calculated by the following formula:

Growth inhibition (%) = $(OD_{control} - OD_{treated}/OD_{control}) \times 100\%$.

The cytotoxicity of sample on tumour cells was expressed as IC_{50} values (the drug concentration reducing by 50% the absorbance in treated cells, with respect to untreated cells), which were calculated by LOGIT method.

4.3. In vivo anti-tumour activity [25]

In vivo anti-tumour activity was evaluated by using a model of transplant sarcoma (S_{180}) and hepatoma 22 (H_{22}) in KM mice, and measuring the index of tumour growth inhibitory rate (%). Briefly, the S_{180} and H_{22} tumour cells were harvested at the exponential growth phase and inoculated subcutaneously into the right flank axilla, then the inoculated mice were randomly divided into groups to receive the solvent control, positive control or one of three different dosages of each compound (**5c**, **6c**, **6h**), with 10 mice per group. The test compounds were dissolved in 0.5% CMC-Na, and the positive drug cyclophosphamide (CTX) was dissolved in normal saline (N.S). The positive control was injected intraperitoneally with CTX (20 mg/kg) and the solvent control was administered intragastrically with an equal volume of 0.5% CMC-Na daily for 10 days. The different compounds were applied at different dosages based on the results of preliminary tests and acute toxicity tests (data not shown). In the S_{180} model, 20, 60 and 180 mg/kg were chosen as the dosages of compounds **6c** and **6h**, and in the H_{22} model, the dosages of 35, 70, 140 mg/kg and 20, 80, 240 mg/kg were chosen, respectively. The dosage of **6c** was 10, 30 and 90 mg/kg in both models. These three compounds were administered intragastrically daily for 10 days. At the end of the experiment, the mice were sacrificed and each tumour was weighed. The tumour inhibition rate (%) was calculated using the following formula:

(Average of tumour weight in the control group − average of tumour weight in the drug-treated group)/average of tumour weight in the control group × 100%.

4.4. Preliminary acute toxicity test

Kunming mice (18–22 g) were divided into three groups for treatment with the three different compounds (**5c**, **6c** and **6h**). The mice were orally administered with 5000 mg/kg in 24 h, and were then monitored continuously for up to 7 days to observe any abnormal behaviour or death. If death occurred, the dosage was decreased and the test repeated.

Acknowledgements

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Appendix. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2011.01.036.

References

[1] M. Decker, J. Med. Chem. 49 (2006) 5411–5413.
[2] M. Calas, M. Ouattara, G. Piquet, Z. Ziora, Y. Bordat, M.L. Ancelin, R. Escale, H. Vial, J. Med. Chem. 50 (2007) 6307–6315.
[3] G.A. Elmegeed, A.R. Baiuomy, O.M. Abdel-Salam, Eur. J. Med. Chem. 42 (2007) 1285–1292.
[4] A.M.S. El-Sharief, Z. Moussa, Eur. J. Med. Chem. 44 (2009) 4315–4334.
[5] L.A. Burke, R.N. Butler, J. Org. Chem. 74 (2009) 5199–5210.
[6] R. Glaser, J. Yin, S. Miller, J. Org. Chem. 75 (2010) 1132–1142.
[7] S.J. Yan, C. Huang, X.H. Zeng, R. Huang, J. Lin, Bioorg. Med. Chem. Lett. 20 (2010) 48–51.
[8] S.J. Yan, H. Zhen, C. Huang, Y.Y. Yan, J. Lin, Bioorg. Med. Chem. Lett. 20 (2010) 4432–4435.
[9] S.J. Yan, Y.J. Liu, Y.L. Chen, L. Liu, J. Lin, Bioorg. Med. Chem. Lett. 20 (2010) 5225–5228.
[10] W.P. Heilman, R.D. Battershell, W.J. Pyne, P.H. Goble, T.A. Magee, R.J. Matthews, J. Med. Chem. 21 (1978) 906–913.
[11] J. Lin, S.J. Yan, L.J. Yang, J.F. Li, F.C. Liu, Chin. J. Org. Chem. 25 (2005) 304–307.
[12] C. Huang, S.J. Yan, Y.M. Li, R. Huang, J. Lin, Bioorg. Med. Chem. Lett. 20 (2010) 4665–4669.
[13] W.K. Hagmann, J. Med. Chem. 51 (2008) 4359–4369.
[14] B.E. Maryanoff, S.O. Nortey, J.J. McNally, P.J. Sanfilippo, D.F. McComsey, B. Dubinsky, R.P. Shank, A.B. Reitz, Bioorg. Med. Chem. Lett. 9 (1999) 1547–1552.
[15] H. Kondo, M. Taguchi, Y. Inoue, F. Sakamoto, G. Tsukamoto, J. Med. Chem. 33 (1990) 2012–2015.
[16] S.N. Suryawanshi, S. Pandey, Rashmirathi, B.A. Bhatt, S. Gupta, Eur. J. Med. Chem. 42 (2007) 511–516.
[17] M.M. Abdelhalim, M.M.T. El-Saidi, S.T. Rabie, G.A. Elmegeed, Steroids 72 (2007) 459–465.
[18] S.J. Yan, C. Huang, C.X. Su, Y.F. Ni, J. Lin, J. Comb. Chem. 12 (2010) 91–94.
[19] CCDC 736001 contains the supplementary crystallographic data for compound 5c. These data can be obtained free of charge from The Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/data_request/cif.
[20] J. Carmichael, W.G. DeGraff, A.F. Gazdar, J.D. Minna, J.B. Mitchell, Cancer Res. 47 (1987) 936–942.
[21] T. Mossman, J. Immunol. Methods 65 (1983) 55–63.
[22] Z.T. Huang, M.X. Wang, Synthesis 12 (1992) 1273–1276.
[23] Z.T. Huang, P.C. Zhang, Chem. Ber. 122 (1989) 2011–2016.
[24] Y.G. Chen, J.J. Xu, H. Yu, C. Qing, Y.L. Zhang, L.Q. Wang, Y. Liu, J.H. Wang, Food Chem. 107 (2008) 169–173.
[25] S.Y. Xu, R.L. Bian, X. Chen, Experimental Methods in Pharmacology. People's Medical Publishing House, Beijing, 2002, pp. 1757–1784.