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The physicochemical properties of a new class of anticancer fungal polysaccharides: A comparative study

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ABSTRACT

The structural and physicochemical properties of polysaccharides isolated from fungi with anticancer properties were investigated. The majority of the polysaccharides considered, have the β-D-Glc*p* component mostly connected by 1→3 and 1→6 linkages in the backbones and the short branches, respectively. The established parameters of *lead-like*, *drug-like* and of known dug space (KDS) were used and the repeating units of the polysaccharides exhibit some overlap with these. It was found that a unique region of chemical space is occupied by the polysaccharides, with MW: 1.0×10^5 to 2.5×10^5 g mol^{-1}; Log *P*: -3.0×10^3 to -1.0×10^3; HD: 1.0×10^3 to 5.0×10^3; HA: 5.0×10^3 to 1.0×10^4; PSA: 5.0×10^4 to 1.0×10^5 and RB: 5.0×10^3 to 1.0×10^4. These findings can be exploited in antitumor drug discovery projects.

1. Introduction

The extracts from medicinal mushrooms are used as prophylactics and for treating diseases (Israilides & Philippoussis, 2003). The therapeutic effects of mushrooms extracts are found for various indications, such as anticancer, antibacterial, antiviral, and antifunal (Ooi & Liu, 1999). One of the potent mushroom-derived bioactive macromolecules are polysaccharides, in particular β-glucan which has anticancer effect by stimulating natural killer cell, T-cells, B-cells, and macrophage-dependent immune system responses through distinct receptors, such as dectin-1, the toll-like receptor-2, scavenger receptors and lactosylceramide (Brown & Gordon, 2005; Dalmo & Bogwald, 2008). For the innate and adaptive immune responses, activated macrophages are responsible for the production of cytokines, interleukin-1 beta (IL-1β), tumour necrosis factor-alpha (TNF-α), nitric oxide (NO), and other inflammatory mediatiors (Porcheray et al., 2005). Compared with many antitumor drugs, the polysaccharides derived from mushrooms are nontoxic and have been consumed since antiquity (Lull, Wichers, & Savelkoul, 2005). Hence, mushroom extracts can be considered as an excellent source for drug candidates. Some fungal polysaccharides are marketed as anticancer pharmaceutical agents in Japan, such as lentinan from *Lentinus edodes*, schizophyllan from *Schizophyllum commune* and krestin from *Coriolus versicolor* (Mizuno, Saito, Nishitoba, & Kawagishi, 1995; Zhang, Cui, Chueng, & Wang, 2007). These polysaccharides and their derivatives have been proved useful against various cancers, especially in the stomach, prostate and lungs (Wasser, 2002).

Definitions of chemical space based on physicochemical parameters are widely used in drug discovery programmes (Hann, 2011; Muchmore, Edmunds, Stewart, & Hajduk, 2010; Reymond, Deursen, Blum, & Ruddigkeit, 2010; Zuegg & Cooper, 2012). There are currently three useful definitions of chemical spaces, i.e., *lead-like*, *drug-like* and *known drug space* (KDS) and the parameters are given in Table 1. The Lipinski's rule of five is the most widely used to define *drug-like* chemical space, which indicates oral absorption of the complying compounds (Lipinski, 1997, 2004). Also the concepts of lead-like chemical space and KDS are used to define useful regions of chemical space. Leads are less complex and have lower molecular weights and lipophilicity (Log *P*) (Oprea, Davis, Teague, & Leeson, 2001). KDS includes all small organic compounds that are in medical use (Bade, Chan, & Reynisson, 2010; Mirza, Desai, & Reynisson, 2009). The idea therefore emerged whether sugar based drugs have a unique region in chemical space, which would enable drug developers to focus their efforts. The aim of this work is to investigate sugar based drugs in clinical use as well as polysaccharides with antitumor properties to establish their region in chemical space, based on main stream molecular descriptors.

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Table 1
Criteria of lead-like, drug-like and known drug space (KDS) in terms of molecular descriptors.

	Lead-like space	Drug-like space	Known drug space
Molecular weight ($g\,mol^{-1}$)	300	500	800
Lipophilicity (Log *P*)	3	5	6.5
Hydrogen bond donors (HD)	3	5	7
Hydrogen bond acceptors (HA)	3	10	15
Polar surface area ($Å^2$) (PSA)	60	140	180
Rotatable bonds (RB)	3	10	17

2. Methodology

The drugs and drug candidates were collected from DrugBank (Knox et al., 2011; Wishart et al., 2008, 2006). To find drugs with sugar moieties, substructure search was conducted using pentose and hexose molecular scaffolds. Three categories, namely pure sugar, modified sugar, and sugar moieties, were used. Six molecular descriptors: molecular weight (MW), Log *P*, hydrogen bond donors (HD), hydrogen bond acceptors (HA), polar surface area (PSA), and rotatable bonds (RB) were utilised and were collected from DrugBank. This information is given in the supplementary information (Table 1).

Mushroom polysaccharides with antitumor properties were found in the literature. Web of Knowledge, ScienceDirect, Scopus and Google Scholar databases were used. The structures of the polysaccharides were drawn in 2D ChemBioDraw Ultra 12.0 (CambridgeSoft, 1986–2009). These structures were converted into 3D objects using the default conversion procedure implemented in the CS ChemBio3D Ultra 12.0 (CambridgeSoft, 1986–2009). The generated structures were optimised using the MM2 (Allinger, 1977) force field with Scigress (Project Leader, Scigress Explorer Ultra, 2000–2007). The QikProp 3.2 (2009) software package was used to calculate the molecular descriptors based on whole molecular predictions. The reliability of QikProp is established for the molecular descriptors used (Ioakimidis, Thoukydidis, Naeem, Mirza, & Reynisson, 2008). A total of 58 polysaccharides exhibiting antitumor ability were gathered from the literature, including 40 polysaccharides from mushrooms and 18 from other natural sources. All the data are given in Table 2.

3. Results and discussion

3.1. Drugs and drug candidates

Overall, 769 drugs and drug candidates were found containing sugar moieties. They comprised of 88 approved and 681 experimental drugs. This is consistent with the study of Bade et al. (2010) who reported that sugar based drugs are relatively uncommon. The categories used were pure sugars, modified sugars, and drugs with sugar moieties. Only 4% (33) are pure sugar and 16% (121) as modified saccharides. Of the drugs found, just over half (52.4%) consist of pentose derivatives, while 5.6% contain both pentose and hexose moieties and the rest (42.0%) has the hexose scaffold. Fig. 1 shows examples of the three categories used.

3.2. The structure of the fungal polysaccharides

The structural attributes of the mushroom antitumor polysaccharides were investigated and the results are shown in Table 3. In general, seven monosaccharide residues including α-D-Glc*p*, β-D-Glc*p*, α-D-Gal*p*, β-D-Gal*p*, α-Man*p*, β-Man*p* and α-L-Fuc*p* are found as the building blocks for the backbone of the polysaccharides. β-D-Glc*p* is the major component which presents the greatest abundance (59%) in the backbone chain. β-D-Gal*p* and α-L-Fuc*p* show the lowest occurrence at just 1%. Those components are connected by four types of linkages, namely 1→2, 1→3, 1→4, and 1→6. The most common linkage is 1→3 at 44%. The monosaccharide units that constitute the branches of the polysaccharides demonstrate a greater diversity as two more monosaccharides are present (α-L-Ara*f*, and β-L-Rha). Similarly, β-D-Glc*p* has the largest percentage in branches at 46%. Besides the four types of linkages found in the backbone, the monosaccharide units also link together through 2→3 substitution (1.8%). The 1→6 linkage is most common in the branches at 54.4%.

The number of branches is apparently one of the important characteristics which significantly affect the antitumor ability (Chihara, Hamuro, Maeda, Arai, & Fukuoka, 1970). When comparing the repeating units of the polysaccharides, different numbers of branches are found to be attached to the backbone of the units. As shown in Table 3, 12.5% of the repeating units do not contain any branches. For the repeating units possessing branches, the numbers of branches are found as only 1, 2, and 4. There is a trend that as the increase in branching, the percentages of their corresponding repeating units decrease. Most repeating units (55.0%) possess only one branch. The rest of the repeating structures hold either 2 or 4 branches. 78% of the repeating units show the branching frequencies from 0.1 (1 in 10 backbone residues) to 0.5 (1 in 2 backbone residues). This range is broader than reported by Miyazaki et al. (1979) who suggested that the optimal branching frequency was from 0.2 to 0.33. Moreover, the polysaccharides exhibiting anticancer activity contain only short branch chains. Taking into account the number of monosaccharide residue, the branches have four types of chains that contain 1, 2, and 3 monosaccharides blocks, respectively. Especially, 86% of them have only one monosaccharide residue to form the branches. Others with short chains of 2 and 3 residues present low percentages.

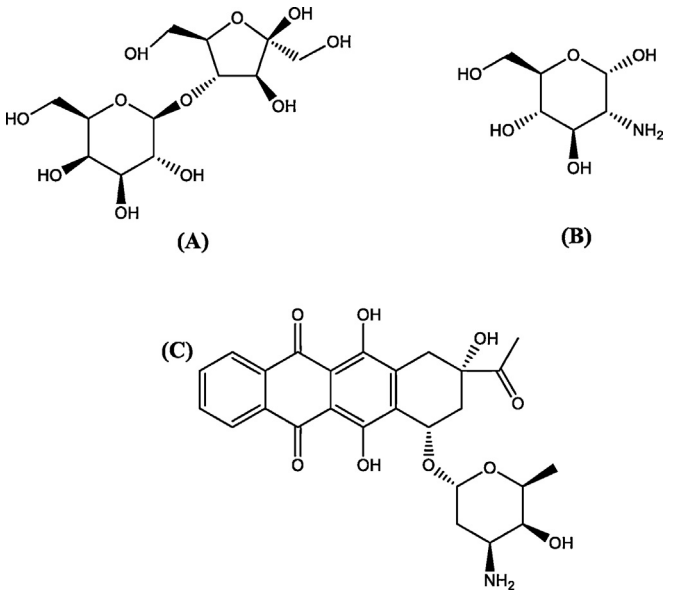

Fig. 1. Structures of lactulose as a pure sugar (A), glucosamine as a modified sugar (B) and doxorubicin as a compound containing a sugar moiety (C).

Table 2
The antitumor polysaccharides used and their different sources.

Source	Polysaccharide fraction	Species	MW ($g\,mol^{-1}$)	Reference
Fungus	Ab2-2N	*Agaricus blazei*	1.7×10^5	Dong, Yao, Yang, and Fang (2002)
	AQS-I	*Astraeus hygrometricus*	2.04×10^5	Chakraborty, Mondal, Pramanik, Rout, and Islam (2004)
	Glucan I	*Auricularia auricula-judae*	1.4×10^6	Misaki (1981)
	AAPS-2	*Auricularia polytricha*	2.59×10^5	Song and Du (2010)
	AAFRC	*Auricularia polytricha*	1.2×10^6	Song and Du (2012)
	PS	*Calocybe indica*	2.0×10^5	Mandal et al. (2011)
	Glucan	*Calocybe indica*	2.0×10^5	Mandal et al. (2012)
	AIPS	*Cordyceps sinensis*	1.15×10^6	Yan, Wang, Li, and Wu (2011)
	WIPS	*Cordyceps sinensis*	1.18×10^6	Yan et al. (2011)
	GLP-2	*Ganoderma lucidum*	2.2×10^4	Li, Fang, and Zhang (2007)
	PSGL-I-1A	*Ganoderma lucidum*	7.18×10^5	Bao, Zhen, Ruan, and Fang (2002)
	GFPS1b	*Grifola frondosa*	2.1×10^4	Cui et al. (2007)
	PS	Hybrid mushroom of *Pleurotus florida* & *Calocybe indica*	2.25×10^5	Maity et al. (2011a)
	PS	Hybrid mushroom of *Pleurotus florida* & *Calocybe indica*	2.01×10^5	Dey et al. (2013)
	PS	Hybrid mushroom of *Pleurotus florida* & *Calocybe indica*	1.98×10^5	Maity et al. (2011b)
	PS-I	Hybrid mushroom of *Pleurotus florida* & *Lentinula edodes*	1.8×10^5	Maji et al. (2012)
	PS-II	Hybrid mushroom of *Pleurotus florida* & *Lentinula edodes*	1.3×10^5	Maji et al. (2013)
	PS-I	Hybrid mushroom of *Pleurotus florida* & *Lentinus squarrosulus*	1.94×10^5	Sen et al. (2013)
	PS-I	Hybrid mushroom of *Pleurotus florida* & *Volvariella volvacea*	1.95×10^5	Patra et al. (2011)
	LDG-A	*Lactarius deliciosus*	1.1×10^4	Ding, Hou, and Hou (2012)
	LT1	*Lentinus edodes*	6.42×10^5	Zhang et al. (2010)
	Lentinan	*Lentinus edodes*	5×10^5	Mizuno (1997)
	PS	*Lentinus squarrosulus*	1.98×10^5	Bhunia et al. (2011)
	PS	*Lentinus squarrosulus*	1.96×10^5	Bhunia et al. (2010)
	PS	*Pleurotus florida*	4.0×10^4	Rout, Mondal, Chakraborty, Pramanik, and Islam (2005)
	PS	*Pleurotus florida*	1.74×10^5	Dey et al. (2010)
	PS	*Pleurotus florida*	1.3×10^5	Roy et al. (2009)
	PS	*Pleurotus florida*	2.5×10^4	Rout, Mondal, Chakraborty, Pramanik, and Islam (2004)
	PS-II	*Pleurotus florida*	1.76×10^5	Dey et al. (2012)
	POPS-1	*Pleurotus ostreatus*	3.1×10^4	Tong et al. (2009)
	POP	*Pleurotus ostreatus*	2.4×10^4	Sun and Liu (2009)
	POPS	*Pleurotus ostreatus*	1.87×10^5	Maity, Patra, et al. (2011)
	SGHW	*Pleurotus sajor-caju*	9.75×10^5	Carbonero et al. (2012)
	PTP	*Polyporus albicans*	3.7×10^4	Sun et al. (2008)
	HCP	*Sarcodon aspratus*	6.7×10^5	Han et al. (2011)
	Schizophyllan	*Schizophyllum commune*	4.5×10^5	Norisuye, Yanaki, and Fujita (1980)
	SCG	*Sparassis crispa*	2×10^6	Tada et al. (2007)
	PS	*Tricholoma crassum*	1.74×10^5	Samanta et al. (2013)
	PS-I	*Termitomyces striatus*	7×10^4	Mondal, Chakraborty, Rout, and Islam (2006)
	PS-I	*Volvariella volvacea*	2.2×10^5	Sarkar et al. (2012)
Plant	PS	*Allium cepa*	1.8×10^5	Patra et al. (2013)
	APS-1d	*Angelica sinensis*	5.1×10^3	Cao et al. (2006)
	APS	*Astragalus membranaceus*	2.07×10^4	Niu et al. (2011)
	AMP-1	*Atractylodis macrocephalae K*	3.8×10^3	Shan, Wei, Deng, and Tian (2003)
	YhPS-1	*Cordalis yanhusuo*	1.2×10^4	Tao and Tian (2006)
	HPS-1B23	*Dendrobium huoshanense*	2.2×10^4	Zha, Luo, Luo, and Jiang (2007)
	DNP-W3	*Dendrobium nobile*	7.1×10^5	Wang, Luo, Yang, and Zha (2010)
	YP-1	*Dioscorea opposita*	4.2×10^4	Zhao, Kan, Li, and Chen (2005)
	HPS-1	*Hedysarum polybotrys*	9.4×10^4	Li et al. (2008)
	PS-I	*Phaseolus vulgaris* L.	1.8×10^5	Patra, Das, Behera, Maiti, and Islam (2012)
	SMPS	*Solenum melongena*	1.92×10^5	Ojha et al. (2009)
Bacterium	EPS	*Bacillus licheniformis* 8-37-0-1	2.83×10^4	Liu et al. (2010)
	PS	*Microellobosporia grisea*	1×10^6	Inoue, Kawamoto, and Kadoya (1983)
	PS1A1	*Mycobacterium bovis*	7.6×10^4	Wang, Klegerman, Marsden, Sinnott, and Groves (1995)
	Clostrhamnan	*Clostridium saccharoperbutylacetonicum*	1.5×10^4	Watanabe, Yamamoto, Misaki, and Hayashida (1987)
Seaweed	F_2	*Capsosiphon fulvescens*	1.2×10^6	Karnjanapratum, Tabarsa, Cho, and You (2012)
Marine animal	ASLP	*Arca subcrenata Lischke*	3.5×10^3	He et al. (2007)
	SEP	*Strongylocentrotus nudus*	1.95×10^6	Liu et al. (2007)

Table 3
Structural information on the repeating units of mushroom antitumor polysaccharides (total polysaccharides = 40).

Backbone component								
α-D-Glc*p*	β-D-Glc*p*	α-D-Gal*p*	β-D-Gal*p*	α-Man*p*	β-Man*p*	α-L-Fuc*p*		
21%	59%	14%	1%	1%	3%	1%		
Branch component								
α-D-Glc*p*	β-D-Glc*p*	α-D-Gal*p*	β-D-Gal*p*	α-Man*p*	β-Man*p*	α-L-Fuc*p*	α-L-Ara*f*	β-L-Rha
23%	46%	4%	4%	4%	5%	7%	5%	2%
Backbone linkage				Branch linkage				
1→2	1→3	1→4	1→6	1→2	1→3	1→4	1→6	2→3
2%	44%	16%	38%	10.5%	15.8%	17.5%	54.4%	1.8%
Branch amount				Amount of monosaccharide in branch				
0	1	2	4	1	2	3		
12.5%	55.0%	30.0%	2.5%	86%	12%	2%		

As the examples of antitumor polysaccharides from mushroom, both lentinan and schizophyllan exhibit branches in their structures. The primary structure of lentinan is a (1→3)-β-glucan containing five (1→3)-β-glucose residues in a linear linkage and two (1→6)-β-glucopyranoside branches in side chains (Fig. 2A). This leads to a right-handed triple helical structure (Chihara, 1992). Schizophyllan is also a (1→3)-β-glucan containing a β-glucopyranosyl group joined by a β-(1→6) linkage to every third or fourth residue of the main chain (Fig. 2B). It has similar triple helix structure and biological activity as lentinan (Ooi & Liu, 2000). β-Glucans exhibit antitumor activity, when they are mostly linear. E.g., pachyman extracted from *Poria cocos* is inactive although it is a branched β-glucan. While, pachymaran that is made of debranching pachyman using periodate oxidation and mild hydrolysis shows pronounced antitumor activity (Chihara et al., 1970).

3.3. Molecular descriptors

Six molecular descriptors were used: MW, Log *P*, HA, HD, PSA, and RB. Their mean, standard deviation and median values were calculated. The results for the drugs and mushroom polysaccharides are shown in Table 4.

3.3.1. Molecular weight

The drugs possessing sugar moieties have average MW of 476.9 g mol^{-1} with standard deviation of 265.4 g mol^{-1} (Table 4).

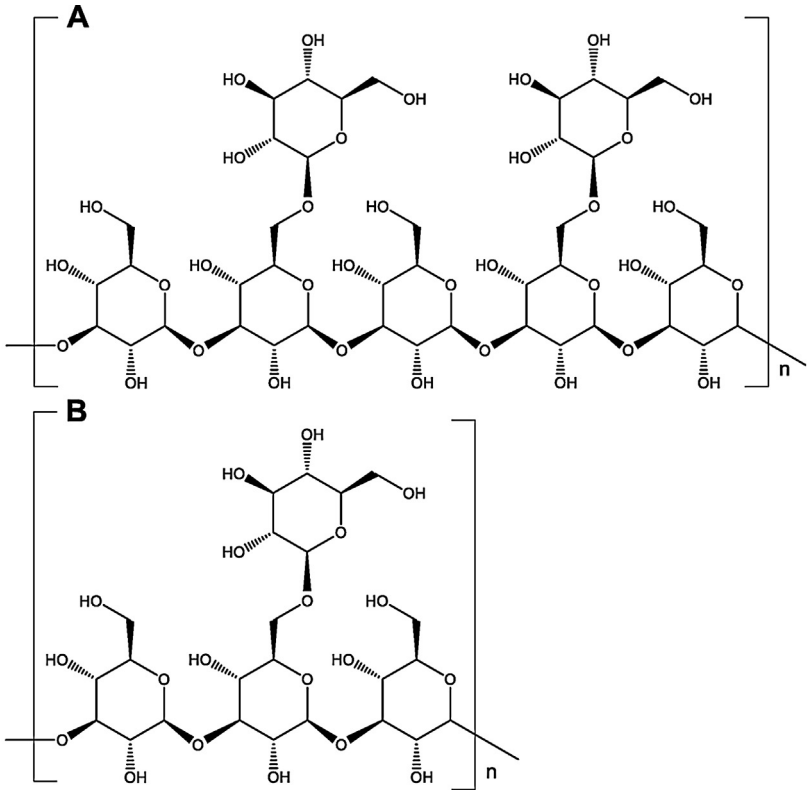

Fig. 2. Structure units of polysaccharides showing antitumor activity: lentinan (A), schizophyllan (B).

Table 4
Mean, standard deviation (STDEV) and median values of the molecular descriptors for different drug types (pure sugar = 33, modified sugar = 121, sugar moieties = 615, total drugs = 769) and antitumor mushroom polysaccharides (polysaccharides = 40).

Compound	Mean	STDEV	Median	Mean	STDEV	Median	Mean	STDEV	Median
Drug type		**Molecular weight**			**Lipophilicity (Log *P*)**			**Hydrogen bond acceptors**	
Pure sugar	334.5	239.5	326.3	−4.3	2.7	−3.8	10.6	7.4	10.0
Modified sugar	310.2	266.0	219.2	−3.3	2.2	−2.7	8.9	7.2	7.0
Sugar moiety	517.4	251.1	454.5	−2.9	3.7	−2.8	11.3	5.1	10.0
Total	476.9	265.4	395.5	−3.0	3.4	−2.9	10.9	5.7	10.0
		Hydrogen bond donors			**Polar surface area**			**Freely rotating bonds**	
Pure sugar	7.5	4.4	7.0	180.2	117.1	169.3	3.4	2.8	4.0
Modified sugar	5.7	3.3	5.0	165.4	136.0	127.5	3.8	5.5	2.0
Sugar moiety	6.3	3.3	6.0	220.1	103.7	192.2	8.5	6.9	7.0
Total	6.2	3.4	5.0	209.8	111.8	178.5	7.5	6.9	6.0
Polysaccharide		**Molecular weight**			**Lipophilicity (Log *P*)**			**Hydrogen bond acceptors**	
Repeating unit	898.2	379.0	824.8	−8.7	4.9	−7.9	44.3	19.7	40.8
Whole molecule	3.8×10^5	4.5×10^5	2.0×10^5	-3.4×10^3	4.1×10^3	-1.8×10^3	1.8×10^4	2.2×10^5	9.6×10^3
		Hydrogen bond donors			**Polar surface area**			**Freely rotating bonds**	
Repeating unit	16.2	6.9	15.0	392.6	159.9	373.6	30.4	13.7	28.0
Whole molecule	6.8×10^3	8.2×10^3	3.6×10^3	1.7×10^5	2.0×10^5	8.7×10^4	1.3×10^4	1.5×10^4	6.5×10^3
		Number of repeating unit							
Whole molecule	542.8	764.9	238.2						

Overall, as illustrated in Fig. 3A, there is a declining trend in the distribution as the value of MW increases. 384 drugs (50%) are concentrated between 100 and 400 g mol^{-1}. Inulin was not included in the pure sugar group to avoid calculating bias since it is known to vary in size. Regarding the three volumes of chemical space defined (Table 1), namely *lead-like* space (MW ≤ 300), *drug-like* space (MW ≤ 500), and KDS (MW ≤ 800), the frequencies of occurrence in the above criteria is 208 (27%) drugs in the *lead-like* space,

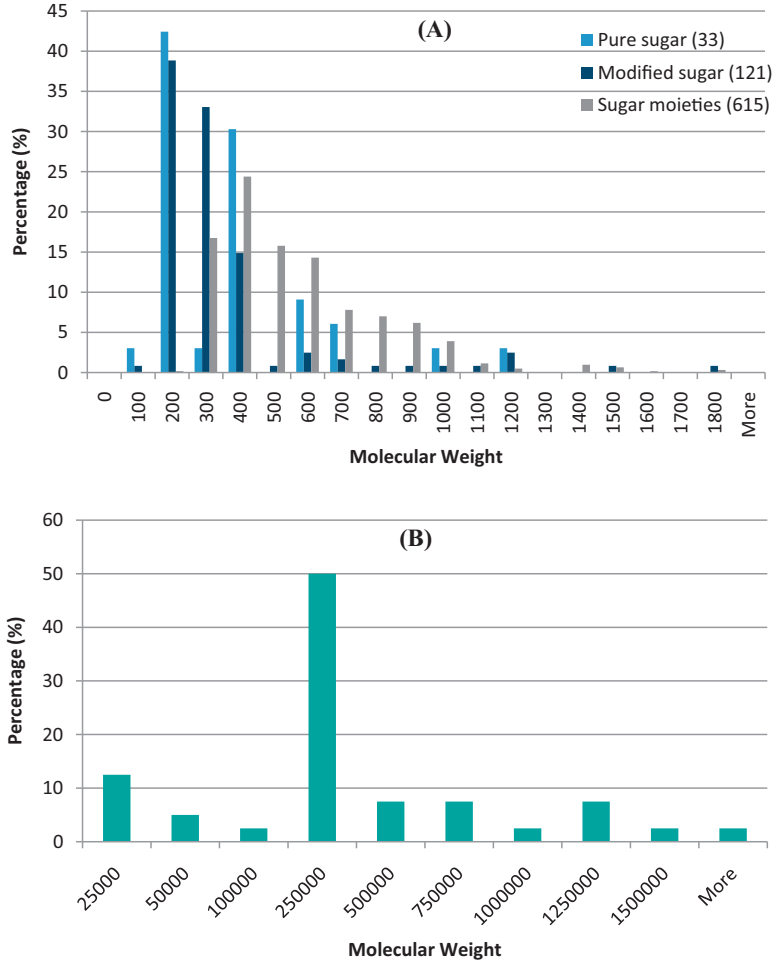

Fig. 3. (A) Distribution of molecular weights of pure sugars, modified sugars, and sugar moieties drugs (total drugs = 769), and (B) mushroom antitumor polysaccharides (polysaccharides = 40).

484 (63%) drugs are in the *drug-like* space, and 674 (88%) are in the KDS. Various studies have indicated that large MW is detrimental to membrane permeation (Wenlock, Austin, Barton, Davis, & Leeson, 2003). However, there is an argument that the definition of *drug-like* chemical space does not apply to saccharides since nutrients are absorbed in the intestine by active transportation (Cundy et al., 2004). E.g., cyclosporine A (MW 1203) is a well-absorbed drug. However, this is difficult to debate due to the lack of evidence of the role of molecular shape on membrane permeability, or the putative role of yet unidentified membrane transporters (Waterbeemd, 2002).

The polysaccharides extracted from mushrooms have a mean MW of 3.8×10^5 g mol^{-1}, which is far larger than those of pure sugar drugs (Table 4). As a result, the polysaccharides do not fit into the three chemical space regions defined in Table 1 for the simple reason that they are macromolecules. As illustrated in Fig. 3B, the majority of the polysaccharides (50%) are in the range from 1.0×10^5 to 2.5×10^5 g mol^{-1} and the remaining polysaccharides are relatively evenly distributed. Natural products often demonstrate good drug properties, which implies that descriptors other than simple MW are more significant for achieving good oral bioavailability (Ganesan, 2008; Lipinski, 2003). Fungal polysaccharides are natural products, which apparently have a unique region in chemical spaces as shown in Fig. 3B.

Based on the information in the current literature the anticancer effect of MW by the polysaccharides can be separated into two routes. On the one hand, it was reported that high molecular weight glucans appeared to be more effective than those with low molecular weight (Mizuno et al., 1996). A (1→3)-β-glucan, with various molecular masses, isolated from the cultured mycelium of *Grifola frondosa* through heat treatment, indicated differences in biological activities. The fraction that had the highest molecular mass (8.0×10^5 g mol^{-1}) showed the most potent antitumor and immunomodulatory activities (Adachi, Ohno, Ohsawa, Oikawa, & Yadomae, 1990). The same effect was seen in a study of four fractions of krestin (polysaccharide–protein complex) with the highest molecular mass fraction showing the strongest immunomodulatory activity (Sakagami, Kim, & Konno, 1990). These studies highlight the possibility that polysaccharides exhibiting antitumor activity may not always be multiple enhancers of the host defence system, and that a high molecular mass is needed for extensive enhancement of immunological and antitumor activities. On the other hand, however, some low molecular weight polysaccharides, such as lentinan and schizophyllan, presented the same antitumor activity against Sarcoma 180 as those with higher molecular weights (Ooi & Liu, 2000). Moreover, unlike (1→3)-β-glucans, Gao, Seljelid, Chen, and Jiang (1996) reported that differences in molecular weight demonstrated no obvious influence on the activity of the heteroglycans. For example, the medical properties of some mushroom polysaccharides like (1→3)-α-glucuronoxylomannans were independent on molecular weight. Their hydrolysate fractions containing glucuronoxylomannans with molecular weights from 53 to 1000 g mol^{-1} were as effective as those fractions possessing higher molecular weights (Gao et al., 1996). In another case, a low molecular weight fraction (MW < 1000) extracted from mushroom *Grifola frondosa* promoted the viability of a human monocyte cell line (THP-1) *in vitro* and increased the production of interleukin (IL)-8, which activated neutrophils since other cytokines produced by monocytes and macrophages such as IL-1, IL-6, IL-10, and IL-12 were unchanged and, finally, TNF-α was not observed (Kodama, Mizuno, Asakawa, Inui, & Nanba, 2010). These conflicting results on the relationship between MW and antitumor activities of different polysaccharides remain to be fully elucidated (Ooi & Liu, 2000). One proposed theory is that the polysaccharides with relatively high MW and expanded chain have more chance to collide and bind more receptors on the surface of immune cells. This boosts the proliferation, differentiation of immune cells, and production of cytokines which are targeted towards destroying tumour cells (Chen, Xu, Zhang, & Zeng, 2009). Another inference is that polysaccharides can lead to apoptosis, programmed cell death, which is modulated by anti-apoptotic and pro-apoptotic effectors involving a great deal of proteins such as the Bcl-2 family (Baea, Janga, Yimb, & Jin, 2005). A low molecular weight α-glucan was reported to cause an apoptosis of HT-29 colon cancer cell by regulating the related proteins inducing programmed cell death (Lavi, Friesem, Geresh, Hadar, & Schwartz, 2006). The polysaccharides from other mushroom species such as *Cordyceps militaris* (Park et al., 2009) and *Angelica sinensis* (Cao et al., 2010) also indicated the induction of the apoptosis of cancer cells. Other published mechanisms related to fungi polysaccharides, such as cell cycle arrest (Hsieh, Kunicki, Darzynkiewicz, & Wu, 2002), necrosis (Guo, Wong, & Cheung, 2011), anti-angiogenesis (Stanley, Harvey, Slivova, Jiang, & Sliva, 2005) and stimulation of immune response (Zhao, Dong, Chen, & Hu, 2010), further confirmed the means and opportunities of polysaccharides against cancer cells. All these mechanisms, and in particular the above cases which involve low MW polysaccharides, provide a hint that the repeating units of the heteroglycans investigated in this study might also show putative antitumor properties.

Since the repeating units of the polysaccharides could hypothetically be the promising candidates of anticancer drugs after depolymerisation, they are tentatively assessed in the chemical spaces using the molecular descriptors. The repeating units of the polysaccharides contain an average value of MW of 898.2 g mol^{-1} (Table 4). None of the repeating units fall into the *lead-like* space, whereas 13% and 30% of them are in the *drug-like* space and KDS, respectively. For the pure sugar drugs, 48% are categorised in the *lead-like* space, 78% in the *drug-like* space, and 94% in the KDS (Fig. 3A).

3.3.2. Lipophilicity (Log *P*)

Log *P* stands for the affinity of a molecule or a moiety for a lipophilic environment (Waterbeemd, 2002), and is often regarded as the most important molecular descriptor related to toxicity issues and failure in clinical trials (Wenlock et al., 2003; Hughes et al., 2008). The majority of the drugs are in the area from −6 to 0, and the distribution for all the three categories present a maximum for Log *P* between −4 and −2 (Fig. 4A). Compared to the other two groups, the sugar moieties group shows a more symmetric distribution as most of them mainly consist of an organic moiety, which makes them relatively hydrophobic. In contrast, as expected, the pure sugar group has a lowest mean value of Log *P* at −4.3 and a standard deviation of 2.7 (Table 4). Nearly all drugs (95%) fall into *lead-like* space (Log $P \leq 3$), while 98% of the drugs are classified in *drug-like* space and 99% of them are in KDS. The average value of Log *P* of the investigated polysaccharides is -3.4×10^3 (Table 4). All the values of Log *P* of the investigated polysaccharides are less than 0, suggesting that they are hydrophilic, i.e., water soluble. 53% of the polysaccharides are dominantly distributed between −3000 and −1000 (Fig. 4B).

When considering the repeating units of the polysaccharides, they have a mean Log *P* value of −8.7, which is somewhat lower than that of pure sugar drugs of −4.3. Owing to their low values, all the repeating units can be assigned into the *lead-like* space similarly to pure sugar drugs as illustrated in Fig. 4. The large Log *P* values generated for the whole polysaccharides are based on the repeating units, which overlap with KDS and are therefore in the range of the predictive power of the algorithm.

3.3.3. Hydrogen bond donors and acceptors

H-bonding is important for describing drug permeability. During transmembrane diffusion, owing to the progressive breakage of

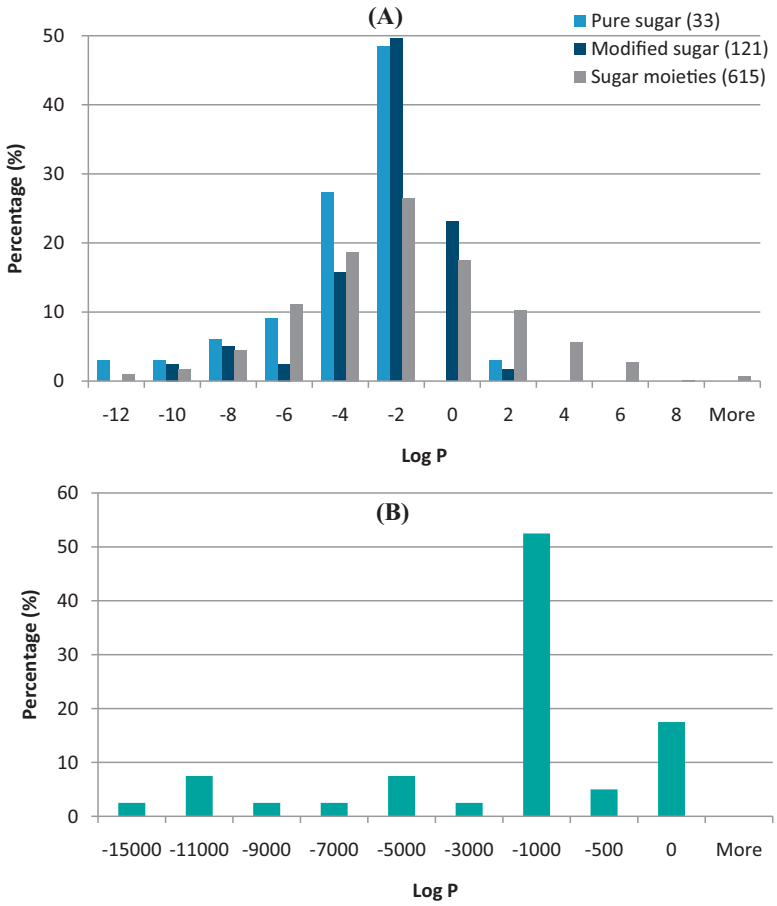

Fig. 4. (A) Distribution of Log *P* values of the pure sugar, modified sugar, and sugar moieties drugs (total drugs = 769), and (B) mushroom antitumor polysaccharides (polysaccharides = 40).

H-bonds, movement of the solute from the region of minimum free energy into the ordered chain region within the bilayer interior is energetically disfavoured (Tejwani, Davis, Anderson, & Stouch, 2011). Table 4 indicates that pure sugar drugs possess the largest mean and standard deviation of hydrogen bond donors at 7.5 ± 4.4 and the second greatest average value of hydrogen bond acceptors at 10.6 ± 7.4. By applying the separating standards of hydrogen bond donors for the definition of chemical spaces, 14% of the drugs are allocated in lead-like space (HD ≤ 3, Fig. 5A). Similarly, hydrogen bond acceptors as illustrated in Fig. 6A, just 3% of the drugs are in lead-like space (HA ≤ 3). Nonetheless, drug-like space embraces 51% and 56% of the drugs based on hydrogen bond donors and acceptors, respectively. For KDS, 72% of the drugs are determined by the standard (≤ 7) of hydrogen bond donors whereas 81% are assigned by that (≤ 15) of hydrogen bond acceptors.

The antineoplastic polysaccharides exhibit high average values of hydrogen bond donors and acceptors at 6.8×10^3 and 1.8×10^4, respectively (Table 4). The number of hydrogen bond donors of the polysaccharides (55%) are mostly located in the region from 1.0×10^3 to 5.0×10^3 (Fig. 5B), while the predominant range of hydrogen bond acceptors are in the area between 5.0×10^3 and 1.0×10^4(48%) (Fig. 6B). With respect to the repeating units of the polysaccharides, their mean values of hydrogen bond donors and acceptors are 16.2 and 44.3, respectively (Table 4). Comparetively, the pure sugar drugs have lower mean values. Concerning the standards of hydrogen bond donors, no repeating units are included in both the *leak-like* and *drug-like* spaces but 13% are in KDS. However, 3%, 45% and 55% of the pure sugar drugs are in the *lead-like*, *drug-like* and KDS spaces, respectively (Fig. 5A). For the boundaries of HA, all repeating units have too large numbers to be in the three chemical spaces, while the pure sugar drugs have 3% in the *lead-like* space, 55% in the *drug-like* space, and 79% in KDS (Fig. 6A).

3.3.4. Polar surface area

The polar surface area (PSA) is defined as the combined surface area that belongs to oxygen and nitrogen atoms and hydrogen atoms bound to these electronegative atoms (Schaftenaar & Vlieg, 2012). As shown in Table 4, the drugs present an average value of 209.8 Å^2 with a standard deviation of 111.8 Å^2. The sugar moiety group has the largest mean value of 220.1 Å^2. According to the definition of PSA, 50% of the drugs fail to be in any of the three chemical spaces (Fig. 7A). Of the drugs, only 1% is in lead-like space (PSA ≤ 60 Å^2), 30% are classified in drug-like space (PSA ≤ 140 Å^2) and 50% in KDS (PSA ≤ 180 Å^2).

The mushroom polysaccharides have a mean value of polar surface area of 1.7×10^5 Å^2 with a standard deviation of 2.0×10^5 Å^2 (Table 4). Likewise, Fig. 7B depicts that most of the values (50%) are found in a narrow region 5.0×10^4 to 1.0×10^5. The average value of PSA of the repeating units of the polysaccharides is 392.6 Å^2 (Table 4). Considering the criteria of PSA, 13% of the repeating units are in KDS but none in *lead-like* and *drug-like* spaces. For the pure sugar drugs, 3% are in *lead-like* space, 45% in *drug-like* space, and 55% in KDS (Fig. 7A).

3.3.5. Rotating bonds

Rotatable bonds are described as any single bond that is bound to a nonterminal heavy atom, but not in a ring (Veber et al., 2002). Generally, the rotatable bond count rises with molecular weight

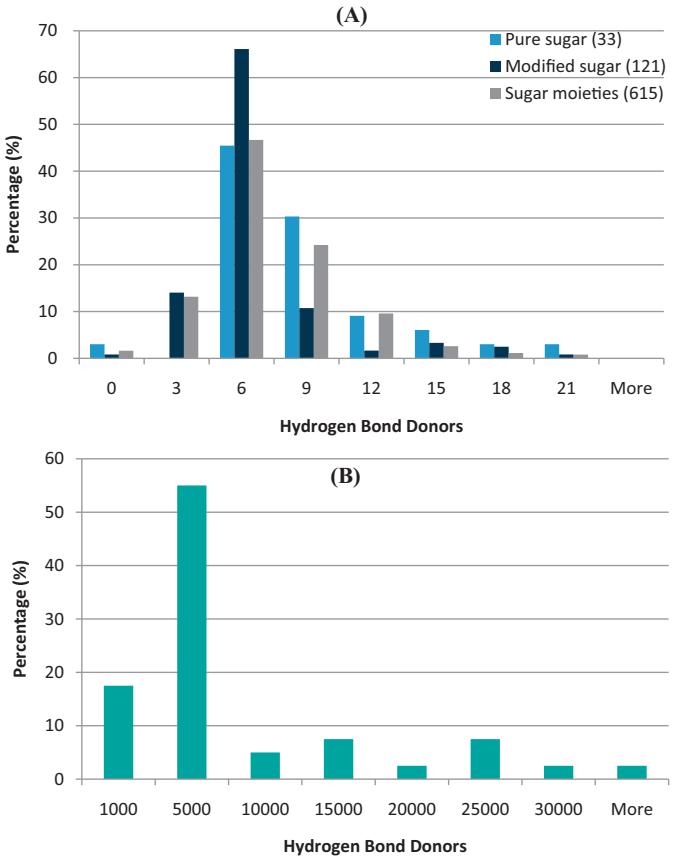

Fig. 5. Distribution of hydrogen bond donors of (A) pure sugar, modified sugar, and sugar moieties (total drugs = 769), and (B) mushroom antitumor polysaccharides (polysaccharides = 40).

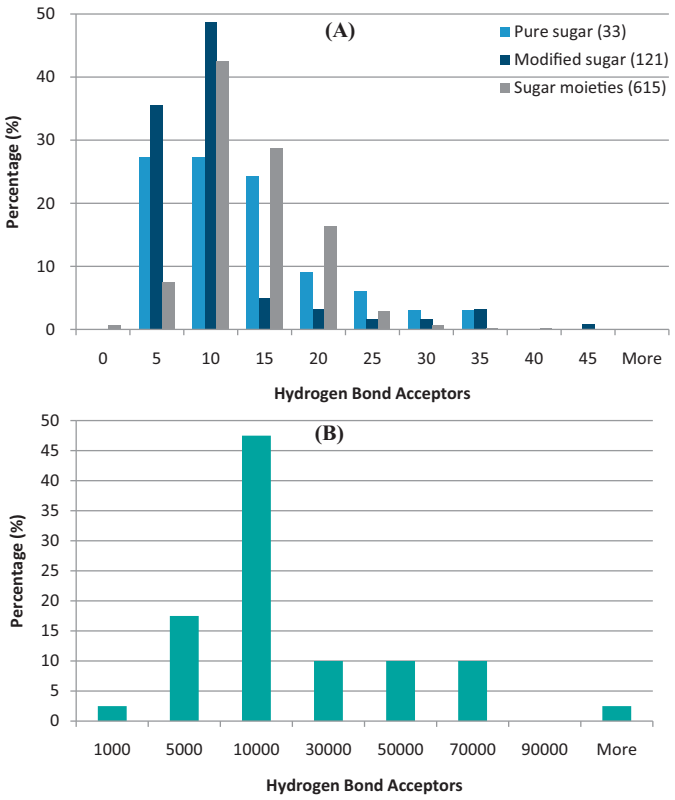

Fig. 6. Distribution of hydrogen bond acceptors of (A) pure sugar, modified sugar, and sugar moieties (total drugs = 769), and (B) mushroom antitumor polysaccharides (polysaccharides = 40).

(Ajay, Walters, & Murcko, 1998). The mean value of rotating bonds of the drugs is 7.5 (Table 4). As shown in Fig. 8A, 29% of the drugs are assigned in lead-like space (RB $\leq$ 3). However, over three-quarter drugs (78%) are classified in drug-like space (RB $\leq$ 10). KDS encompasses 93% of the drugs. The average value of RB of antitumor polysaccharides is 1.3×10^4 (Table 4). As shown in Fig. 8B, 48% of the polysaccharides have the values of rotatable bonds from 5.0×10^3 to 1.0×10^4. The repeating units of the polysaccharides possess a mean value of RB of 30.4. Based on the boundaries of RB in chemical space, the *drug-like* space and KDS contain the close percentages of the repeating units at 13% and 16%, respectively, but the *lead-like* space fail to involve any of them. In the case of the pure sugar drugs, 48% are in the *lead-like* space and 100% are in *drug-like* space and also in KDS (Fig. 8A).

3.4. Polysaccharides with antitumor properties from other sources

In order to investigate the different structural characteristics of the antitumor properties of polysaccharides from mushrooms and other sources, eighteen were collected from the literature. Specifically, eleven polysaccharides are from plants, four from bacteria, two from marine animals and one from algae as shown in Table 2. Compared with the constituents in the backbone of mushroom polysaccharides, three new monosaccharide residues (β-Fru*f*, α-L-Ara*f* and α-Rha) are found in these polysaccharides but α-L-Fuc*p* that is seen in mushroom polysaccharides is absent. For the components in the branches, eight monosaccharide residues, including α-Glc*p*, β-Glc*p*, α-Gal*p*, β-Gal*p*, α-Man*p*, β-Man*p*, α-Ara*f*, β-Rha, are found in common in the two groups of polysaccharides.

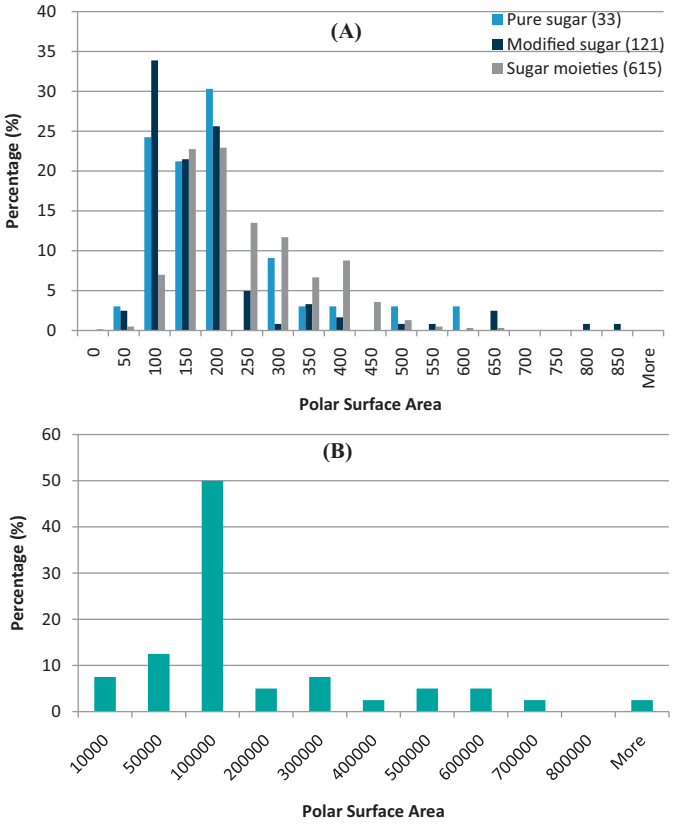

Fig. 7. Distribution of polar surface area values of (A) pure sugar, modified sugar, and sugar moieties drugs (total drugs = 769), and (B) the mushroom antitumor polysaccharides (polysaccharides = 40).

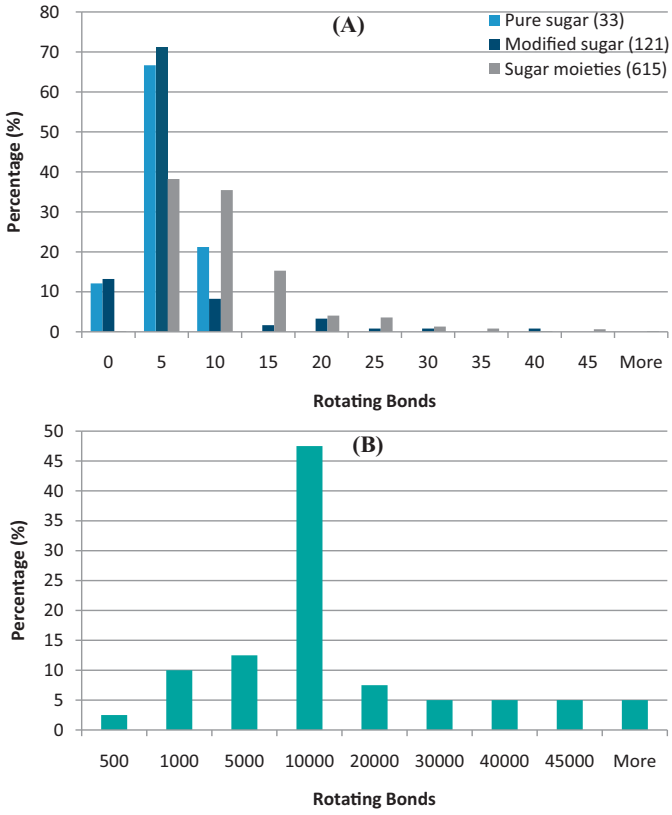

Fig. 8. Distribution of freely rotating bonds of (A) pure sugar, modified sugar, and sugar moieties drugs (total drugs = 769), and (B) mushroom antitumor polysaccharides (polysaccharides = 40).

However, there are still two distinct differences that α-L-Fuc*p* can only be observed in the mushroom polysaccharides, while β-Fru*f* and β-L-Ara*f* are only found in the non-mushroom polysaccharides. Also, unlike the mushroom polysaccharides having β-Glc*p* as the most common component, α-Glc*p* is the most abundant component found in the backbone (65%) and branches (40%) of the polysaccharides from other sources. Discrepancy is also found in the linkage patterns. The backbone of the non-mushroom polysaccharides possess two new glycosidic linkages 1→5 (1%) and 2→6 (5%). The dominant linkage type in the backbone of the mushroom polysaccharides is 1→3 at 44%, while overwhelming linkage type in non-mushroom ones is 1→4 (61%). Similarly, for the difference in the branches, non-mushroom polysaccharides show a scarcity of 2→3 linkage. Furthermore, both groups of the polysaccharides have the most abundant linkage type 1→6 in the branches, 54.4% for the mushroom and 52% for non-mushroom. Again, most of the non-mushroom polysaccharides (68%) only have one monosaccharide unit in their branches. Nonetheless, non-mushroom polysaccharides contain longer branch chains composed of five (4%) or six (4%) monosaccharide units, which are not in mushroom polysaccharides.

In general, the polysaccharides derived from other resources than mushrooms have lower mean values for most molecular descriptors except for Log *P*. The mean values are as follows: MW = 2.6×10^5; Log *P* = -2.5×10^3; HD = 4.5×10^3; HA = 1.3×10^4; PSA = 1.0×10^5; RB = 8.7×10^3; and the number of the repeating unit is 247.0. The reason for mushroom polysaccharides exhibiting higher values is due to their longer polymer chains with an average number of the repeating unit of 542.8. Regarding the repeating unit of the non-fungal polysaccharides MW, none are in the *lead-like* and *drug-like* spaces but 28% lie in the KDS. For Log *P*, all repeating units fall into all the three chemical spaces due to their negative values.

In summary, mushroom and non-mushroom polysaccharides differ by composition and conformation. The non-mushroom polysaccharides demonstrate a greater diversity in the molecular structures as they contain more types of monosaccharide residues in both backbones and branches. Despite of containing fewer constituent types, mushroom polysaccharides have one more glycosidic linkage type than their non-mushroom counterparts. The molecule size of mushroom polysaccharides is normally larger due to more repeating units. Although the backbone chains of mushroom polysaccharide molecules are longer, non-mushroom polysaccharides have longer branch chains. All these differences indicate that the fungal polysaccharides' antitumor properties could be based on different biochemical mechanisms.

4. Conclusions and suggestions

This is the first study to investigate the physicochemical properties of antitumor polysaccharides by integrating individual researching outcomes from the literature. Known sugar based drugs were used as a reference to the polysaccharides regarding the chemical space occupied by these two cohorts. In general, the sugar based drugs are small molecules and the polysaccharides are macromolecules, which therefore present much larger values for the main stream molecular descriptors. However, the repeating units of the polysaccharides show some overlap with *lead*, *drug-like* and KDS. A clear preference was seen for the polysaccharides to occupy a certain volume in chemical space, i.e., MW: 1.0×10^5 to 2.5×10^5 g mol^{-1}; Log *P*: -3.0×10^3 to -1.0×10^3; HD: 1.0×10^3 to 5.0×10^3; HA: 5.0×10^3 to 1.0×10^4; PSA: 5.0×10^4 to 1.0×10^5 and RB: 5.0×10^3 to 1.0×10^4. This region and the particular characteristics in the compositional structure could be applied to hunt new drug candidates based on polysaccharides. Furthermore, this profile could also be a preliminary indication of the correlation between the anticancer mechanism and the physicochemical properties of the sugars. Finally, mushroom sugars are in many respects unique compared to polysaccharides from other natural sources even though both have anticancer properties.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.04.064.

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