PHARMACOPHORE MODELLING FOR THE DISCOVERY OF SYSTEM X_C^- ANTIPORTER INHIBITORS

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ARTICLE INFO	ABSTRACT
	Cancer is one of the major disorders with increasing rates of morbidity and mortality. Recent drug discovery of anti cancer drug has identified several molecular targets and tried to achieve a goal of therapeutic effecative and safe molecule. Amongst these, system x_c^- antiporter is a novel promising target to control cancer progression. This antiporter is found to be over expressed in majority of cancer cells and functions by transporting amino acids, cystine and glutamate, in opposite directions. System x_c^- antiporter uptakes one molecule of cystine with the release of one molecule of glutamate in extracellular space. As already known cystine is precursor for the synthesis of glutathione, an in vivo antioxidant which is utilized by cancer cells to combat oxidative stress. At the other side the released glutamate (an excitatory neurotransmitter), when released in higher concentration, may over excite neurones (specifically and brain tumour) causing cell death to metastasise cancer cells. Therefore, through inhibition of system x_c^- antiporter, it is possible to kill cancer cells by disturbing their redox status along with through prevention of excitotoxcity by glutamate. In context to this, several researches have reported diverse molecules having system xc- antiporter inhibition potential. Amongst these molecules, erastin and its analogues are most potent system xc- antiporter inhibitors but it lacks preclinical data. Moreover, sulfasalazine, a FDA approved drug also showed good inhibition potential against this antiporter and therefore in our study we have attempted to construct pharmacophore model using this series to aid in the discovery of potent inhibitors with desirable safety. Results of this study exhibited successful development of pharmacophore model with phase survival score. Additionally, fit scores of sulfasalazine analogues were also in acceptable range. Hence, the developed pharmacophore model may be used for design of potent System xc- antiporter inhibitors.

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INTRODUCTION

System x_c^- antiporter is an anionic amino acid transporter present on number of cells within body [1]. The prime function of System x_c^- antiporter is to uptake one molecule of cystine with release of one molecule of glutamate in equal concentrations [2]. Thus, it helps in maintaining redox status of cancer cells through continuous supply of glutathione (GSH) [3]. Additionally, in CNS, system x_c^- antiporter supports nerve conduction through the release of an excitatory neurotransmitter glutamate [4]. Structurally, system x_c^- antiporter is made up of two polypeptide chains viz. heavy chain (4F2hc) and light chain (xCT). The main functional characteristic of system x_c^- antiporter is associated with light chain while the heavy chain is required for trafficking of xCT [5].

There is ample generation of reactive oxygen species (ROS) in cancer cells due to altered metabolic state which may cause oxidative stress. Therefore, cancer cells need to have protective mechanism. In this regard, cancer cells over express system x_c^- antiporter which helps in maintaining redox status and ultimately protect cancer cells. Several in vitro experiments reported that system x_c^- antiporter is over expressed in cancer cell lines like glial [7], head and neck [8], lung [9], breast [10], gastrointestinal [11], pancreatic [12], ovarian [13] and colon [11]. Therefore, system x_c^- antiporter is an important target for the treatment of above motioned cancer.

The system x_c^- mediated amino acid transport is a rate limiting step for production of glutathione (GSH) in cancer cell lines thereby inhibition of system x_c^- antiporter may throw cancer cells off-balance in their redox environment, causing apoptosis due to excessive ROS production. Two FDA-approved drugs, sulfasalazine (SSZ) and sorafenib are potent inhibitors of system x_c^-. Additionally, erastin was shown to have the highest potency amongst all system xc- inhibitors investigated till date [13, 14]. Sorafenib, a conventional molecularly-targeted anticancer drug, is associated with lot of adverse effects while erastin lacks promising in vivo anticancer data. Moreover, the enzymatic breakdown of SSZ in gut makes it poorly bioavailable [15]. Furthermore, many researchers have synthesized derivatives of amino methylisoxazole propionic acid (AMPA) and amino-3-carboxy-5-methylisoxazole propionic acid and analogues of SSZ to evaluate their system x_c^- inhibitory activity [15]. Amongst these inhibitors, SSZ, an approved anti-inflammatory drug, is a promising lead for further chemical optimization for potency and pharmacokinetic properties since it inhibits system x_c^- with moderate potency and it lacks the adverse effects associated with approved anticancer drugs. However, major limitations of SSZ include its very poor systemic bioavailability (approximately 12%), its rapid cleavage by colonic bacteria into inactive constituents and short half-life (~80 min). Its BBB permeability is also not known.

Ligand based pharmacophore modelling is a tool which helps in identifying the chemical attributes required by related any molecules to have ligand specific biological activity. Since already mentioned earlier, we still lack the clinically available system xc-antiporter inhibitor for the treatment of variety of cancers. Therefore our main objective was to construct pharmacophore model and finally use the same for designing novel System xc- antiporter inhibitors.

MATERIALS AND METHODS
Selection of ligands

Structurally diverse molecules with their respective system x_c^- antiporter inhibition potential have been reported in earlier studies. However, we only used sulfasalazine analogues for pharmacophore modelling due to its reported safety in humans.

Ligand preparation

The molecular structures sulfasalazine and its anlogues were drawn using chemdraw office suite ultra v 9.0 software (CambridgeSoft Corp., UK). Schrödinger Release 2016-1: LigPrep [21] was used to assign appropriate ionization states, ring conformations, and stereoisomers. Finally energy minimization of all the molecules was performed before constructing pharmacophore model.

Ligand based pharmacophore modelling

The pharmacophore model was generated using sulfasalazine analogues using Schrödinger Release 2016-1. The common pharmacophore hypothesis was obtained through PHASE followed by tree based partitioning algorithm having maximum tree depth of four. This was performed to recognize general pharmacophoric feature to generate the possible model. The default setting with terminal box of 1Å was used to generate common pharmacophore. A set of SMARTs was used to represent internal pharmacophore site. Briefly, three possible geometries defined the physical characteristics of sites where site is either located on single atom OR the site is located on a single atom as like point, but it will be assigned based on one or more vectors originating from that atom and according to directionality OR the site is located on the group of atoms at the centroid. So, four point pharmacophore model was generated where the pharmacophore includes two Hydrogen bond acceptor, one Hydrogen bond donor and one aromatic ring.

Scoring

To examine common pharmacophore hypothesis, scoring function was used. Overall maximum root mean square deviation (RMSD) value of 1.2 A° was used to obtain the better alignment of the ligands. Quality of alignment is measured by survival score.

RESULTS AND DISCUSSION

Sulfasalazine and its analogues were used to develop a pharmacophore model. Sulfasalazine moiety contains an aromatic amine attached to the diazo linker on left side and two aromatic ring having sulphonamide as a linker on right side. As mentioned earlier, we selected for points to develop hypothesis and considering these features in all molecules, number of models were generated. Amongst these several hypothesis, we selected three hypothesis based on survival score (Table 1).

Table 1: Three best pharmacophore hypotheses along with their respective phase survival scores.

Sr. no	Model	Phase survival score
1	AADR.2	2.51
2	AADR.1	2.71
3	AAAR.27	2.77

Additionally, we also carried out PLS analysis by keeping grid spacing 1 Å to derive three regression models. The best fitted model- I was AADR.1 with phase survival score 2.51 (Table 1). The pharmacophore hypothesis for model-I is depicted in Figure1 where hydrogen bond donor (D) are shown in light blue sphere centred on H atom, with an arrow pointing in the direction of potential H bond. Hydrogen bond acceptors (A) are shown in light red sphere centred on the atom with the lone pair, with arrow pointing in the directions of lone pairs. The aromatic ring is shown in orange torus in the plane of the ring.
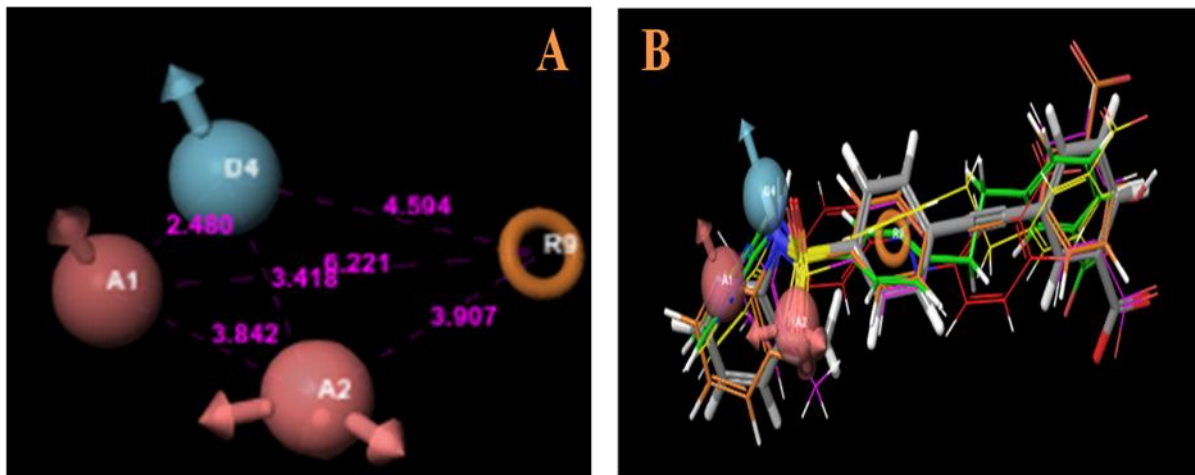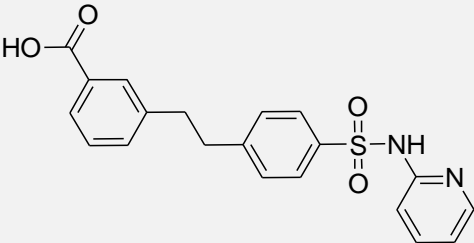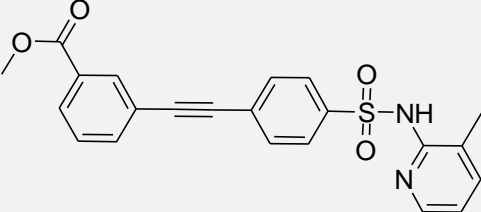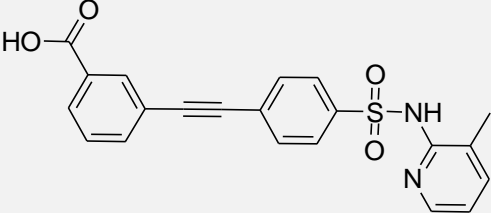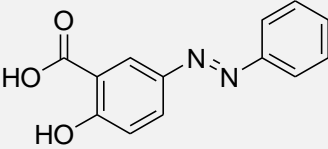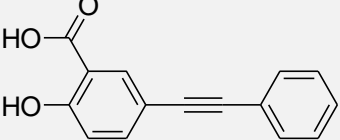

Figure 1. A) Pharmacophore distances between pharmacophoric sites, B) The common pharmacophore based alignment of sulfasalazine analogues.

According to the best hypotheses (Figure 1A), the distance between one H- bond acceptor (A1) and H-bond donor (D4) should be 2.48 Å while the H-bond acceptor (A1) and aromatic ring (R) should be 6.22 Å far from each other. The distance between two hydrogen bond acceptor (A1 and A2) and A1 to R should be 3.84 and 3.9 Å, respectively. Moreover, the distance between D4 and R1 should be around 4.59 Å. We have also shown the alignment of sulfasalazine and analogues, which defines the basic pharmacophore (AADR model) requirement for system x_c^- inhibition (Figure 1B). Additionally, we also calculate the fit score of each participating molecule (Table 2).

Table 2: Fit score of the sulfasalazine analogues when aligned with pharmacophore template.

Sr. No	Molecule name	Molecule structure	Fit score
1	Sulfasalazine 6		2.5
2	Sulfasalazine 6A		2.64
3	Sulfasalazine 7		2.58
4	Sulfasalazine 8		2.05
5	Sulfasalazine 9		2.96

CONCLUSION

We successfully developed pharmacophore model using sulfasalazine and its analogues. The bottom line of our research is that, the generated pharmacophore model has acceptable phase survival score and hence can be used for designing novel system xc-inhibitors in future studies. The developed inhibitors through this model with proven biology can be further used to refine the model and then again used to develop more potent compounds.

Conflict of interest
Authors declare no conflict of interest.

List of abbreviations
FDA   : Food and drug administration
ROS   : Reactive oxygen species
4F2hc   : Heavy chain of system xc- antiporter
Xct   : Light chain of system xc- antiporter
GSH   : Glutathione
SSZ   : Sulfasalazine
AMPA   : Amino methylisoxazole propionic acid
BBB   : Blood brain Barrier
RMSD   : Root mean square deviation
PLS   : Partial least square

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