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Synthesis, Antimicrobial Activity and Molecular Modelling of Aminolysed Derivative of Poly(ethyleneterephthalate)

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ABSTRACT

The increasing demand and use of Poly(ethylene terephthalate) (PET) in different applications have led to serious concern for our environment. Poly(ethylene terephthalate) has to be recycled to compounds of significant importance by chemical or mechanical method to reduce the hazards created by this non-biodegradable waste. In this report, we have prepared bis(2-aminoethyl terephthalamide) (BAET) from aminolysis of PET in presence of ethylene diamine. The basic characterization of the compound was done by using FTIR, UV-visible spectroscopy, NMR and thermal analysis studies. In addition to this, the antimicrobial approach of the compounds obtained through recycled PET was examined which showed broad spectrum antibacterial and anti-fungal property. The present study also includes molecular docking of synthesized compound with the target protein GlcN-6-P synthase which explains a promising activity of this compound as an antimicrobial agent. In silico study reveals that BAET had high affinity towards the target protein by showing good binding energy ranging between -8.55 to -7.45 kcal/mol^{-1}. Compound BAET has very closed interactions with target protein's Cys300, Gly301, Ser303, Leu601, Glu488, Ser349, Ser347, Gln348, Lys603, Ser401, Ala400, Leu601 and Glu488.

Keywords: Poly(ethylene terephthalate), ethylene diamine, aminolysis, antifungal activity, antibacterial activity, molecular docking, target protein

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INTRODUCTION

Poly(ethylene terephthalate) (PET) is a thermoplastic polyester which has the characteristic of non-toxicity, high durability, non-biodegradability and transparency. These all characteristics make it useful in many applications such as in textiles clothing, photographic films, and drinking bottles. In present scenario the use of PET has drastically increased and its non-biodegrability has led to the problem of waste disposal [1]. Keeping in mind the environmental concern, efforts are being made to recycle the PET and to avoid the dumping of waste. There are two main conventional methods for PET recycling: mechanical recycling and chemical recycling [2,3]. By mechanical shearing PET generally converts into low grade product as fibre. Chemical recycling mainly involves depolymerization through hydrolysis, methanolysis, and glycolysis [4-7].

Soni and Singh [8] have suggested degradation of PET by aqueous methylamine and ammonia in presence of catalyst. Tawfik and Eskander [9] have investigated PET degradation with ethanolamine in presence of dibutyl tin oxide (DBTO) as a catalyst. In presence of other catalyst such as glacial acetic acid, sodium acetate and potassium sulphate with ethanolamine, PET was depolymerised [10]. By the reaction of aliphatic amines like butylamine, hexylamine, octylamine in excess amount, PET was degraded and terephthalic diamides were prepared. Spychaj and coworkers [11] described the chemical degradation of PET with other polyamines such as diethylenetriamine, triethylenetetramine and their mixtures [11]. The recycling of PET with ethanolamine gave product bis(2-hydroxyethyl) terephthalate (BHETA) which was used to synthesize polyurethane [12]. The terephthalic diamides

were used as stabilizers for LDPE [13]. PET degradation in presence of hydrazine monohydrate generated terephthalic dihydrazide which have been used as secondary plasticizers in PVC compounding [14,15].

Several studies have been explored to study the drug potency of the recycled compounds [16]. Drug designing and molecular docking helps to understand ligand protein interaction and provides practical information regarding prediction of binding orientation of small molecule candidate to their protein. Ligand is small molecule and can interact with binding sites of protein through various possible mutual conformation called binding modes [17]. An attempt was made to study molecular docking by using target protein L-Glutamine: D-fructose-6-phosphate amidotransferase (EC2.6.1.16) known by trivial name of glucosamine-6-phosphate synthase (GlcN-6-P synthase) [18]. This enzyme involve hexosamine metabolism, which converts D-fructose 6-phosphate (Fru-6-P) into GlcN6P in the presence of glutamine as an ammonia source and finally form an N-acetylglucosamine (NAG). NAG is important for cell wall formations in bacteria and fungi, such as peptidoglycan in bacteria and chitin, mannoproteins in fungi. In human beings, N-acetylglucosamine is used for biosynthesis of glycoproteins and mucopolysaccharides. Infact in all kind of cells the GlcN-6-P synthase is present [19]. In the prokaryotic cell, the inactivation of GlcN-6-P synthase even for a short time is lethal. But, the depletion of amino sugar pool for a short time is not lethal in mammalian cell due to the longer lifespan of mammalian cells, long half life time of Glu-6-posphate synthase and fast expression of mammalian gene encoding the enzyme Glc-6-phosphate synthase [20]. This difference in the metabolism of the enzyme has made GlcN-6-P synthase an important target for drug discovery.

In the present work, the aminolysis of PET waste was carried out using ethylene diamine (EDA) and end product was investigated and characterized as bis-amino ethylterephthalamide (BAET). The basic characterization of the compound was done by using FTIR, UV-visible spectroscopy, NMR and thermal studies. In addition to this, the antimicrobial approach of the the compounds obtained through recycled PET was examined which showed broad spectrum antibacterial and anti-fungal property. The present study also includes molecular docking of synthesized compound with the target protein GlcN-6-P synthase which explains a promising activity of this compound as an antimicrobial agent.

MATERIALS AND METHODS

Chemicals - PET waste flakes were collected by cutting of mineral water bottles. PET flakes were rinsed in boiling water and washed with acetone and dried in oven at about 70 ^{o}C. Glucose and sodium chloride were purchased from Merck and Fluconazole, Yeast extract, Peptones were purchased from HiMedia.

Culture Maintenance-

Fungus species- *Aspergillus niger Aspergillus flavus, Aspergillus fumigates, Pennicillium sp., Candida albicans, Rhizopus* and *Mucor* were grown in our laboratory on SDA (Sabouraud Dextrose Agar) media and streptomycin antibiotic.

Bacteria species- *E. Coli* and *Bacillus cereus* were procured from department of Microbiology, C.C.S University, Meerut. They were grown on the Nutrient Agar medium in the Biochemistry Laboratory at C.C.S University, Meerut.

Recycling of PET waste: Aromatic amides were recycled through degradation of PET waste by using ethylene diamine [23]. 10 gm of PET waste flakes were mixed with 100 mL of ethylene diamine in a reagent bottle and left undisturbed at ambient conditions of temperature and pressure for seven days. Reaction scheme has been given in **Fig. 4.** After seven days, white coloured powder BAET was obtained and filtered by vacuum pump. The compound was further washed with distilled water. After two or three washes the soluble part was filtered and insoluble part was obtained and dried under vacuum oven at a temperature of 50 °C. The solid material was insoluble in methanol or acetone. BAET was subjected for characterization through FTIR, UV, NMR, TGA-DTA, and DSC for structural analysis.

Characterization-The UV-Visible spectra of compound was recorded in DMSO. Cleared filtrate was read by using double beam Perkin Elmer UV-Vis spectrophotometer λ25 at the 2880 nm/min scan speed in the range of 190 nm to 800 nm at data interval of 10 nm. Cycle time and number of cycle set were 0.1 second and 4. FTIR spectroscopy was recorded on ATR module of Cary 630 FTIR, Agilent Technologies, at Department of Chemistry, C.C.S. University, Meerut. NMR Spectra was recorded on Bruker at Jammu University, Jammu, for the ultimate degraded alcoholised product obtained. BAET was mixed in tolerated DMSO and exposed to magnetic field using trimethylsilane (TMS) as a standard. Differential scanning calorimetry (DSC) was performed using METTLER STARe SW 8.10 instrument. BAET was heated from -50 ^{o}C to 500 ^{o}C with heating rate of 10 ^{o}C/min in nitrogen

atmosphere. Thermogravimetric analysis (TGA-DTA) was done on Perkin Elmer STA 6000 at a heating rate of 20 ºC/min from 40 ºC to 730 ºC.

Antifungal Activity: BAET was screened for antifungal activity against the following fungal species *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus Pennicillium, Candida albicans, Rhizopus* and *Mucor*. Different concentrations 2 mg/mL, 20 mg/mL, and 40 mg/mL of BAET were prepared to determine zone of inhibition of fungal growth. The petridish was casted with media and well was introduced with the help of sterile cork borer (8 mm in size). The test compound dissolved in DMSO was filled in these wells. The antifungal activity of compound was compared with Fluconazole whereas antibacterial activity of test compounds was compared with Ciprofloxacin [23,24]. Fluconazole and Ciprofloxacin are used as standard antimicrobial agent [25]. Fungal strains *Aspergillus niger, Aspergillus flavus, and Aspergillus fumigates* were exposed to different concentrations of BAET disssolved in DMSO to determine the zone of inhibition. *Aspergillus niger* was also tested for minimum inhibitory concentration of amide in liquid media.

Antibacterial Activity-The synthesized BAET was screened against two bacteria Gram positive bacteria *Bacillus cereus* and Gram negative bacteria *E. coli* using Ciprofloxacin (Cipla, India) as reference material. Antibacterial activity was defined in term of measured zone of inhibition diameter keeping DMSO as blank control.

***In silico* Molecular Docking**- The ligand (BAET) was drawn in ChemSketch. Energy of molecule was minimized using by PRODRG server [26]. After energy minimization the compound was read as input for AutoDok 4.2, in order to carry out the docking simulation [27] using Lamarckian genetic algorithm. After conducting adequate review Glucosamine-6-phosphate synthase (PDB ID 2VF5) was selected as the target protein for the present study. The heteroatoms were removed from the 2VF5.pdb to make complex receptor free of any ligand before docking. The graphical user interface program "AutoDock tools" was used to prepare, run and analyse the docking simulation. During the docking, the grid dimensions were 126 x 126 x 126 Å with point separated by 0.375 Å. Grid centre was set as 26.638, 22.694, 8.036 for x, y, and z respectively. Lamarkian Getetic Algorithm was used as the docking algorithm with 10 runs, 150 population size, 2,500,000 maximum number of energy evaluation, and 27,000 maximum number of generation. RMSD cluster analysis was performed using the ligand atoms only (24/24 total atoms).

RESULTS AND DISCUSSION

Characterization of BAET- BAET was subjected for characterization through FTIR, UV, NMR, TGA-DTA, and DSC for structural analysis. The characteristic UV absorption band of aromatic amide was observed at 250 nm suggesting presence of carbonyl group and aromatic ring. FTIR spectrum was shown in Fig.1(a). The absorption band at 3292 cm^{-1} showed presence secondary amide, 1628 cm^{-1} showed stretching vibration of C=O of amide bond. Out of plane hydrogen deformation for para substituted benzene appears at 857 cm^{-1}. ^{1}H NMR of BAET is shown in Fig.1(b). The single peak at 7.9 ppm (4H) are characteristic for asymmetrical para-substituted aromatic ring. The two sets of peaks at 8.6 and 8.7 ppm showed –NH protons. The two sets of peaks observed at 3.3 and 3.5 ppm are produced by the two sets of methylene hydrogen atoms attached to the amide and amine groups respectively.

Thermal Properties of BAET-The melting point and thermal decomposition of BAET was investigated by TGA-DTA and DSC. Fig. 2(a) showed the TGA-DTA thermogram showing about loss of 15.1 wt% in between 170-250 °C and 70 wt% loss between 240 -400 °C. Fig. 2(b) showed DSC graph of BAET. The melting point of BAET is 190 °C and melting energy is 272 J/gm. The decomposition of compound begins at 171°C, which is very near to melting point and continues to reach a broad peak at 250 °C. The reports from Dutta and Soni have supported the use of BAET in epoxy resin as curing agent due to its good thermal resistance property. The kinetics of BAET monomer with epoxy resin as curing agent was studied by DSC isothermal method which showed lower energy of activation, 10.65 kJ/mol at high temperature [21].

Antifungal Activity- Antifungal activity was determined against fungal species viz., *Aspergillus niger Aspergillus flavus, Aspergillus fumigatus Pennicillium, Candida albicans, Rhizopus* and *Mucor* at the amide concentration 2 mg/mL, 20 mg/mL and 40 mg/mL. The activity was shown in terms of diameter of zone of inhibition in Table 1.0. Inhibition zone were compared between the different *Aspergillus* species at different concentration viz., 50, 40, 35, 30, 25, 20, 15, 10, 5 mg/mL of amide as described in Table 2.0.

Aspergillus niger growth was found to be inhibited in the presence of synthesized compound BAET at every serial dilution viz., 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.125 mg/mL, 1.565 mg/mL in broth culture (Table 3.0). On prolonged incubation of more than 48 hrs very small vegetative fungal growth was seen in the aromatic amide at concentration of 3.125 mg/mL. Only vegetative growth and no spore formation was seen. At a very low

concentration of 0.15 mg/mL, fungal growth with spore formation was seen. The minimum inhibitory concentration was found to be 3.125 mg/mL at 48 hrs. The results suggested that in liquid culture minimum inhibitory concentration for BAET decreased to 1.5 mg/mL in comparison to well diffusion method (15 mg/mL) for *Aspergillus niger*.

Table 1.0: Analysis of zone of inhibition of different concentration of BAET against different fungal species (Fluconazole as standard and DMSO as blank)

	Zone of inhibition (mm)						
	Aspergillus niger	***Aspergillus flavous***	***Aspergillus fumigates***	***Pennicillium***	***Rhizopus***	***Mucor***	***Candida albicans***
BAET (2 mg/mL)	0	10	4	0	0	4	0
BAET (20mg/mL)	4	20	12	4	4	12	8
BAET (40mg/mL)	14	22	17	7	8	17	14
Fluconazole (2 mg/mL)	0	6	2	0	4	0	8
DMSO	0	0	0	0	0	0	0

Table 2.0: Comparative study for inhibition of different *Aspergillus* species at different concentration of amide (Fluconazole as standard and DMSO as blank)

	Zone of inhibition (mm) at BAET Concentration (mg/mL)										
	50	**40**	**35**	**30**	**25**	**20**	**15**	**10**	**5**	**Fluconazole**	**DMSO**
Aspergillus niger	14	14	12	14	10	6	4	0	0	0	0
Aspergillus flavus	20	22	22	22	20	20	20	20	17	16	0
Aspergillus fumigatus	17	17	12	10	12	12	10	10	7	0	0

Table 3.0: Minimum Inhibitory concentration of BAET against *Aspergillus niger.*

S. No.	Microorganism	Concentration	Observation after 24 hours	Observation after 48 hours	Observation after 72 hours
1	*Aspergillus niger*	50 mg/mL	No growth observed	Total inhibition	Total inhibition
2	*Aspergillus niger*	25 mg/mL	No growth observed	Total inhibition	Total inhibition
3	*Aspergillus niger*	12.5 mg/mL	No growth observed	Total inhibition	Total inhibition
4	*Aspergillus niger*	6.25 mg/mL	No growth observed	Total inhibition	Total inhibition
5	*Aspergillus niger*	3.125 mg/mL	No growth observed	Total inhibition	Small vegetative growth
6	*Aspergillus niger*	1.5625 mg/mL	No growth observed	Total inhibition	Vegetative growth with spore formation
7	*Aspergillus niger*	DMSO Control	No growth observed	Normal growth	Normal Growth

Table 4.0: Antibacterial activity of bis (2-aminoethyl) N, N' terephthalamide (BAET)

S. No.	Bacteria	Concentration (mg/mL)	Zone of inhibition (mm)
1	*E. coli*	10 mg/mL	10
2	*E. Coli*	5 mg/mL	8
3	*E. Coli*	2 mg/mL	6
4	*E. coli*	1 mg/mL	4
5	*E. Coli*	DMSO	No inhibition
6	*E. coli*	Ciprofloxacin (2 mg/mL)	32
7	*B. cereus*	10 mg/mL	4
8	*B. cereus*	5 mg/mL	4
9	*B. cereus*	2 mg/mL	1
10	*B. cereus*	1 mg/mL	1
11	*B. cereus*	DMSO	0
12	*B. Cereus*	Ciprofloxacin (2 mg/mL)	26

Table 5: Molecular docking parameter of BAET with Glucosamine-6-phosphate synthase

S. No.	Run	Rank	Binding energy (kcal mol^{-1})	Inhibition constant (µM)	Final Intermolecular energy (kcal mol^{-1})
1	5	1	-8.55	0.543	-10.93
2	7	2	-8.08	1.20	-10.46
3	9	2	-7.81	1.87	-10.2
4	10	2	-7.75	2.10	-10.13
5	8	2	-7.45	3.46	-9.84
6	4	3	-7.96	1.47	-10.34
7	2	4	-7.82	1.84	-10.21
8	6	4	-7.33	4.25	-9.71
9	3	5	-7.56	2.88	-9.95
10	1	6	-5.22	148.45	-7.61

Antibacterial Activity- The synthesized BAET has shown antibacterial activity against *Bacillus cereus* and *E. coli* described in Table 4.0. It has shown good zone of inhibition in presence of higher concentration of BAET. *E. coli* growth was inhibited even at 1 mg/mL concentration whereas *Bacillus cereus* growth was inhibited at 5 mg/mL concentration.

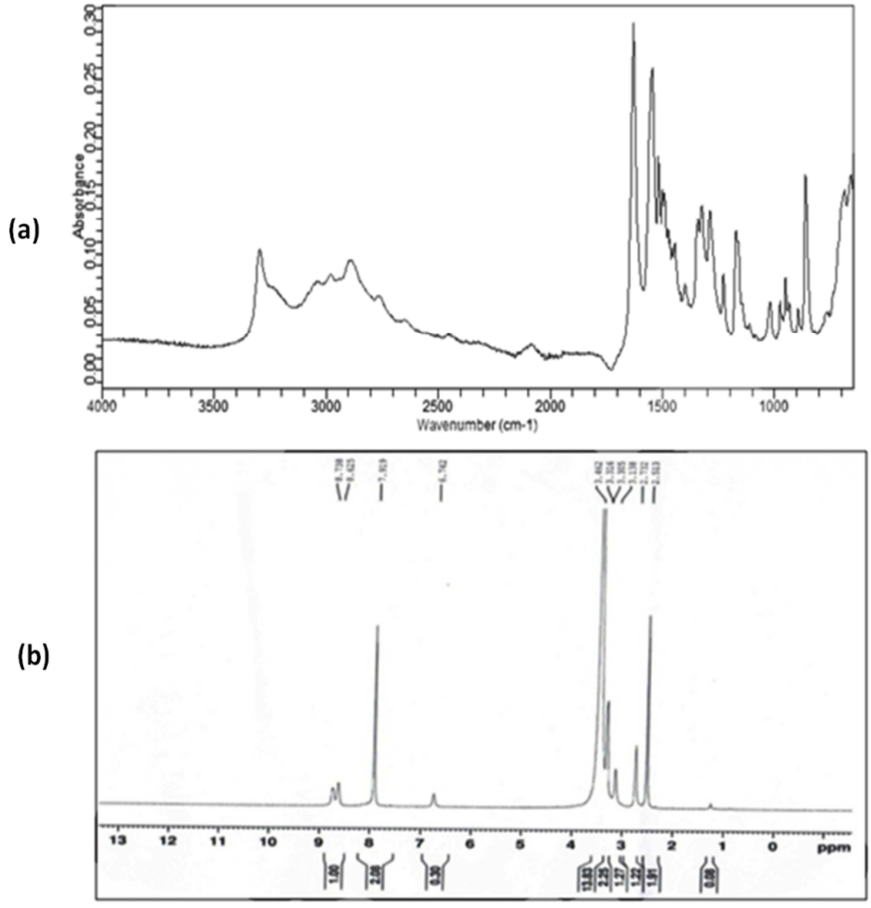

Fig. 1. (a) FTIR (b) ^{1}H NMR spectrum of bis (2-aminoethyl) N, N' terephthalamide (BAET)

Molecular Docking Studies- After obtaining well *in vitro* antimicrobial activity of synthesized compound, the study of molecular docking was performed. The synthesized compound was docked inside the active site of Glucosamine-6-phosphate synthase, the potential target for antimicrobial and antifungal agents. X-ray study of Glucosamine-6-phosphate synthase (PDBID: 2VF5) reveals 12 amino acids in active pocket as Ala602, Val399, Ala400, Gly301, Thr302, Ser303, Cys300, Gln348, Ser349, Thr352, Ser347 and Lys603. Fig.3(a) shows 3D structure of Glucosamine-6-phophate synthase [22]. Docking studies are computational techniques to explore possible binding modes of a ligand to a given receptor. In this study, AutoDoc 4.2 was used to evaluate the binding energy of ligand inside the active site of enzyme. Lamarkian Genetic Algorithm (LGA) was used for docking analysis, it gave 10 docked conformations ranking according to their binding and intermolecular energies. Docking parameters were predicted by clustering histogram such as binding energies, inhibition constant (Table 5.0). Fig. 3(c) indicates the binding of the best generated conformers for the compound. The high ranking binding energy of the generated conformer was -8.55 kcal/mol^{-1} and lowest inhibition constant was 0.543µM. *In silico* study reveals

that BAET showed high affinity by good binding energy towards the target protein ranging -8.55 to -7.45 kcal/mol^{-1}. In the seventh run energy difference of the docked complex is very less and there is formation of two hydrogen bonds with lowest binding energy of -8.08 kcal/mol^{-1}. In this conformation, compound BAET has very closed interactions with target proteins of Glucosamine-6-phosphate synthase Cys300, Gly301, Ser303, Leu601, Glu488, Ser349, Ser347, Gln348, Lys603, Ser401, Ala400, Leu601 and Glu488.

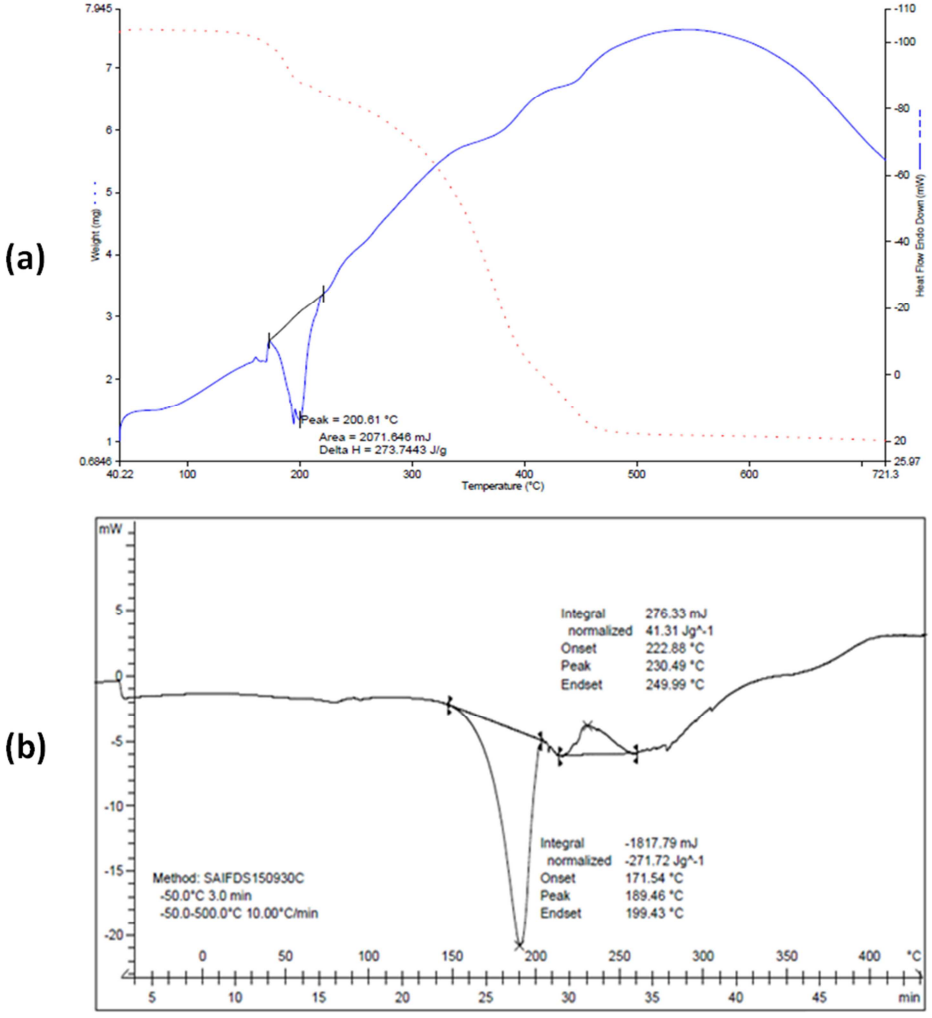

Fig. 2. (a) TGA/DTA and (b) DSC of bis (2-aminoethyl) N, N' terephthalamide (BAET)

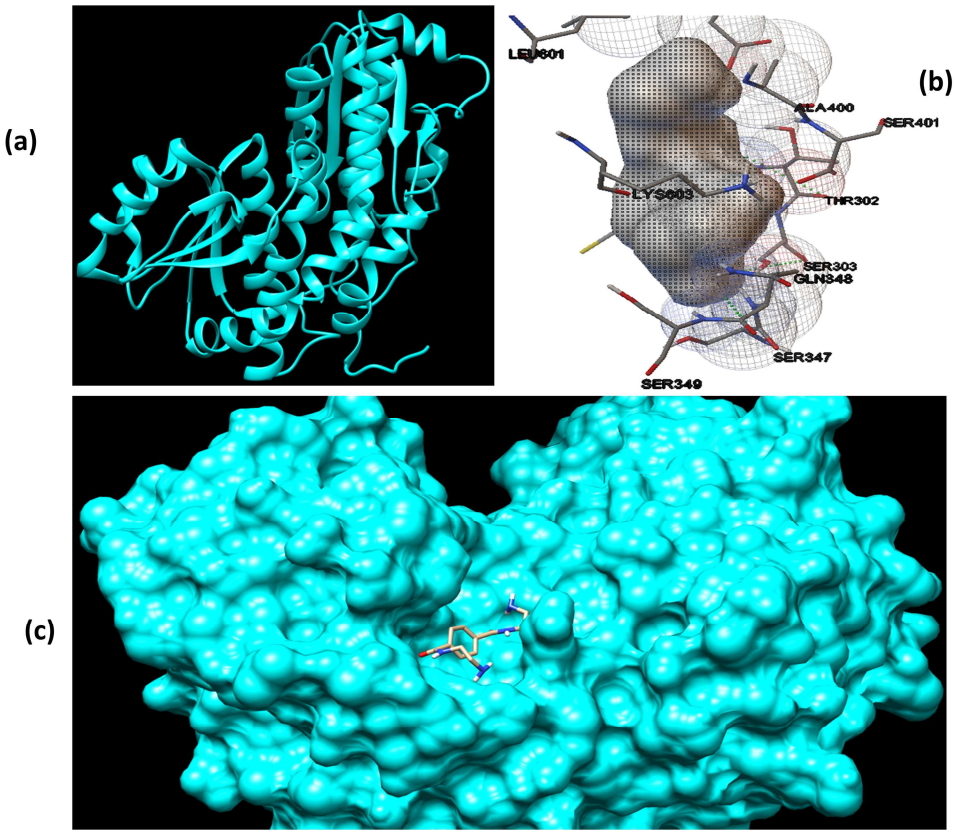

Fig. 3. (a) X-ray crystallography structure of Glucosamine-6-phosphate synthase, (b) Autodoc final interaction between ligand and protein Glucoasmine-6-phosphate synthase, (c) chimera docked in best conformation

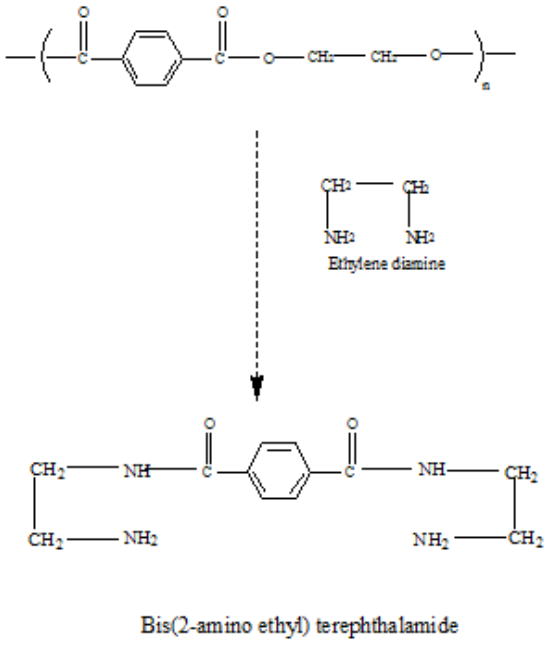

Fig. 4. Representation of preparation of bis(2-aminoethyl) N, N' terephthalamide from PET flakes in presence of ethylene diamine

CONCLUSION

The non-biodegradable PET waste is of no importance but its aminolysed derivative bis(2-aminoethyl terphthalamide) prepared herein showed broad spectrum antibacterial and antifungal properties. BAET was prepared from PET by aminolysis in presence of ethanol diamine. It was characterized by FTIR, NMR and UV-visible spectroscopy for structural analysis. The thermal data suggest that BAET shows good thermal resistance. Antifungal activity was determined against fungal species viz., *Aspergillus niger Aspergillus flavus, Aspergillus fumigatus*

Pennicillium, Candida albicans, Rhizopus and *Mucor* at different concentrations and the activity was expressed in terms of zone of inhibition. Antibacterial activity against *Bacillus cereus* and *E. coli* has shown good zone of inhibition in presence of higher concentration of BAET. *E. coli* growth was inhibited even at 1 mg/mL concentration whereas *Bacillus cereus* growth was inhibited at 5 mg/mL concentration. The synthesized compound was docked inside the active site of Glucosamine-6-phosphate synthase, the potential target for antimicrobial and antifungal agents. The high ranking binding energy of the generated conformer was -8.55 kcal/mL^{-1} and lowest inhibition constant was 0.543µM. *In silico* study reveals of BAET showed high affinity by good binding energy towards the target protein ranging -8.55 to -7.45 kcal/mol^{-1}. Compound BAET has very closed interactions with target protein Cys300, Gly301, Ser303, Leu601, Glu488, Ser349, Ser347, Gln348, Lys603, Ser401, Ala400, Leu601 and Glu488.

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