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ARTICLE TYPE

Novel carbapenem chalcone derivatives: Synthesis, cytotoxicity and molecular docking studies

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One pot efficient synthetic protocol is described for the synthesis of carbapenem chalcone derivatives using AAPTMS@MCM-41 heterogeneous catalyst. Various substituted aromatic aldehydes were attached to highly chiral and reactive carbapenem using this approach. The cytotoxic activity evaluation of all synthesized compounds was performed against lung cancer cell lines (A-549) and breast cancer cell lines (MCF-7 using the MTT assay. Among the tested compounds, compound CPC-2 showed better activity against MCF-7 cell lines with IC_{50} value 2.52 µM/mL; whereas compound CPC-4 showed good activity against A-549 cell lines with IC_{50} value 1.59 µM/mL. In order to support the observed activity profiles, the representative compounds were flexibly docked into the active sites of the Anaplastic Lymphoma Kinase (ALK) enzyme and the Estrogen receptor (ERβ). The most active anti-cancer compounds exhibited stronger binding affinities for the proteins

Hybrid approach is a promising path to develop drugs that can target diseases more effectively; these are the compounds with different structures and variety of biological activities. Novel biological activities can be gained distinct from the ones of components by optimizing properties like affinity and selectivity, right balance of the properties can be achieved through the hybrid approach. Hybrid entities are just like double edged swords with multiple pharmacophore units.[1-3] Most of the clinically used compounds contain *β*-lactams, and are extensively used as anti-bacterial agents, *β*-lactamase inhibitors and in other therapeutic areas.[4] *β*-Lactams perform their functions as trans peptidases-and *β*-lactamase inhibitors. Carbapenem and aztreonam are amongst the *β*-lactams which can be synthesized, due to high chirality and reactive bicyclic ring structure synthetic modification of these compounds makes the task challenging.

Chalcones are the small non-chiral molecules with *α*, *β* unsaturated carbonyl group. They are central cores of several biologically active compounds exhibiting interesting biological properties like antioxidant, cytotoxic, anticancer, antimicrobial, antiprotozoal, antiulcer, antihistaminic and anti-inflammatory activities.[5-14] Many lead compounds were developed earlier due to their diverse biological properties; for these reasons chalcones become a tool of enthusiasm both in academia and as well as in industry. Biological diverse properties of the two molecules encouraged us towards the diamine functionalized mesoporous silica meditated synthesis, biological investigation and docking studies of carbapenem chalcones derivatives.

Review of the literature for chalcone synthesis revealed many techniques and strategies like Aldol condensation, Claisen-Schmidt condensation, Suzuki reaction, Witting reaction, Friedel-Crafts acylation with cinnamoyl chloride and Photo-Fries rearrangement of phenyl cinnamates etc. among all the available methods, the Claisen-Schmidt condensation, however, still holds the apex position. Conventionally Claisen-Schmidt condensation reaction is carried out using catalysts like alkaline bases,[15] $Ba(OH)_2$,[16]and LiOH,[17] recently a new range of catalysts like $SOCl_2$, natural phosphate, lithium nitrate, amino grafted zeolites, zinc oxide, water, Na_2CO_3, PEG-400, silica sulfuric acid, $ZrCl_4$, Mesoporous Zirconium Phosphate, Mesoporous AlSBA-15-SO_3H Hybrid Material, Silicotungstic acid, Sulfated Degussa Titania, Alkaline-doped Carbons, Novel Solid Sulfonic acid from Bamboo, Aminopropylated Silica Sol–gel and ionic liquid etc.[18] were reported for the synthesis of chalcone derivatives. Given the structural variation of our starting material carbapenem there is always a scope for the development of a new synthetic strategy, though the A ring contributor carbapenem of the hybrid molecule derivatives contains active methyl keto group its connection to highly chiral and reactive bicyclic ring structure makes the synthesis the interesting. The literature review reveled that amine functionalized mesoporous silica promotes addition and condensation reaction like Michael and Aldol,[19] these reports encouraged us to synthesize a new catalyst i.e. diamine functionalized mesoporus silica (AAPTMS@MCM-41) as the present synthesis proceeds via Claisen-Schmidt condensation; we successfully employed the catalyst for the present synthesis.

Here in we describe the synthesis of carbapenem chalcone derivatives and their efficiency in *in-vitro* anticancer activity against two cancer cell lines using the cisplatin as a reference

drug.

The structure of AAPTMS@MCM-41was characterized using the FT-IR spectroscopic method, and its spectrum is shown in **figure 1**. In this spectrum, the band at 1080-1090 cm^{-1} was due to Si-O starching of Si-O-Si structure. The band at 1620-1640 cm^{-1} due to H-O-H bending vibration of H_2O and 3100-3600 cm^{-1} for absorption of water molecule.[20] The band at 690 cm^{-1} due to N-H bending vibration and the band at 1532 cm^{-1} was due to $–NH_2$ symmetric bending vibration. The band at 2935 cm^{-1} indicating CH_2 groups of the propyl chain of the silylating agent of the materials.[21]

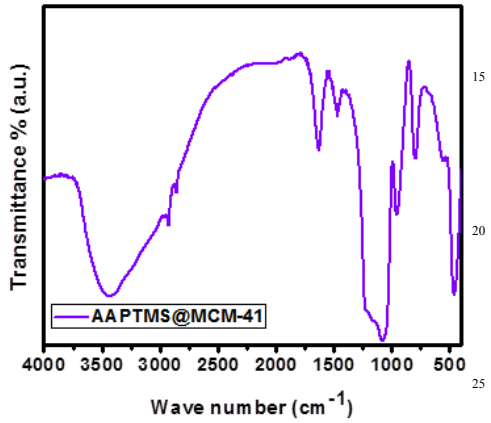


Figure 1. FT-IR spectra of AAPTMS@MCM-41

The PXRD patterns of the samples were obtained on Rigaku D/Max III VC diffractometer with Cu Kα radiation at 40 kV and 40 mA in the range of 2θ = 0-80°. The FE-SEM was performed with a ZEISS 55 microscope. The TEM images were viewed on a Jeol JEM-1010 electron microscope.

The small angle & high angle XRD patterns of AAPTMS@MCM-41 are shown in Figure 1A &1B. In this materials display a strong peak at 2θ = 2.2° due to (100) plane and also small peaks indicate the formation of well-ordered mesoporous materials due to higher order (110), (200) and (210) plane reflections within 5°. Only the little bit reduction of the (100) peak. So, the mesoporosity remains unchanged after the modification of the silica network by both organo groups. The high angle XRD spectrum shows (**Figure 2)** only dispersion of organic group in a non-crystalline form on the surface of MCM-41.

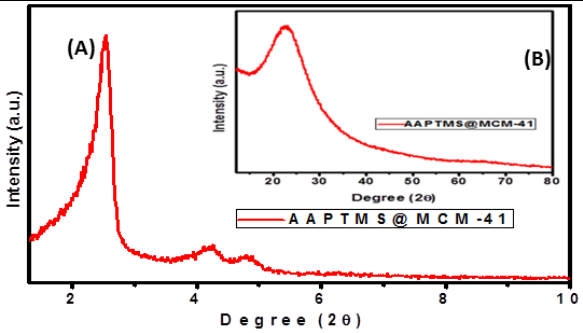


Figure 2. (A) Small angle XRD pattern of AAPTMS@MCM-41; (B) High angle XRD pattern of AAPTMS@MCM-41 sample

The Scanning electron microscopy of AAPTMS@MCM-41 is shown in the **figure 3**. In this image shows slightly elliptical morphology. Thus it was conformed that, the particles are well order after modification of organic group on the support surface parent material. The EDX spectrum of **figure 4** gives the information about AAPTMS@MCM-41sample. In this image only, N, Si, C and O present in this materials.

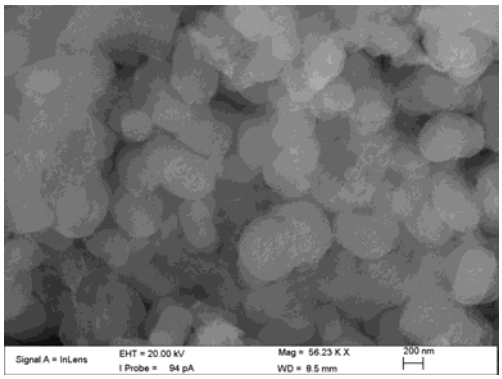


Figure 3. SEM image of AAPTMS@MCM-41 sample

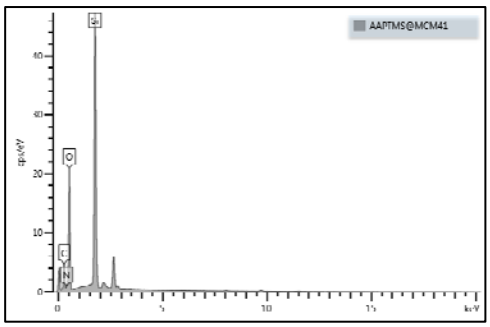


Figure 4. EDX spectra of AAPTMS@MCM-41 sample

The transmittance electron microscope (TEM) image of AAPTMS@MCM-41 (**figure 5**), it was conformed that after modification the materials exhibit well-ordered elliptical morphology. The high magnification image shows that the material is porous nature.

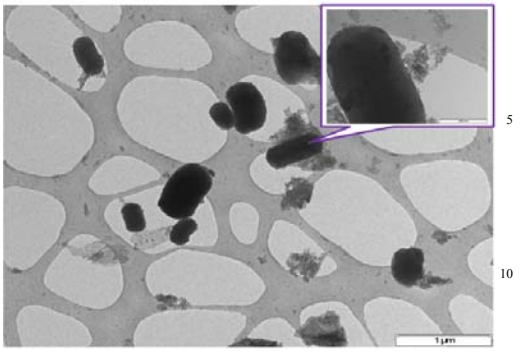


Figure 5. TEM image of AAPTMS@MCM-41

Optimization of reaction conditions and identification of best base catalyst were achieved by varying the chosen catalysts and solvents successively (**Scheme.2**). Initially the reaction between carbapenem and substituted aryl aldehydes was examined using conventional catalysts like KOH/EtOH, NaOH/EtOH, Silica sulphuric acid and $Ba(OH)_2$, having failed to achieve the desired products in good amounts we employed base catalyst AAPTMS@MCM-41 yielded desired products with more than 90% yield within less than 10 minutes of reaction time.

Table 1. Optimization of reaction conditions

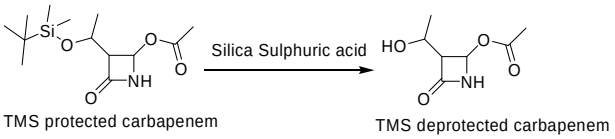
Entry	Catalyst	Amount	Solvent	Time (h)	Yield [a](%)
1	NaOH	-	$EtOH/H_2O$	9	20
2	NaOH	-	ACN	9	30
3	KOH		$EtOH/H_2O$	9	25
4	KOH	-	ACN	9	35
5	SSA		$EtOH/H_2O$	9	20
6	SSA		ACN	9	25
7	$Ba(OH)_2$		$EtOH/H_2O$	9	15
8	$Ba(OH)_2$		ACN	9	20
9	Catalyst[b]		$EtOH/H_2O$	9	60
10	Catalyst[b]		ACN	8min	92

[a]Isolated yields; [b]AAPTMS@MCM-41.

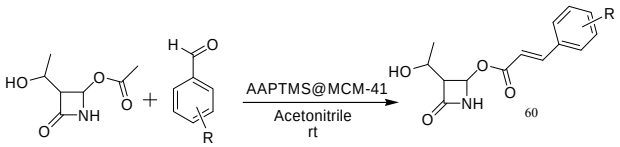
SSA: Silica Sulfuric Acid

Note: Reactions performed in room temperature (27°C ± 3)

The basic cites in catalyst AAPTMS@MCM-41 helped in the condensation between active methyl groups of carbapenem and substituted aromatic aldehydes (**Scheme 2**). The catalyst was recovered by simple filtration. Activity of the catalyst was retained up to 5th cycle. Studies with recycled catalyst showed that it can be reused at least four times with no significant loss (<4%) of activity (**Table 2**). The activity loss observed with the regenerated catalyst later could be due to partial loss of base sites or surface area and the activity diminished due to some surface contamination of the catalyst during reaction/regeneration. Elemental and spectral (IR, ^1H, ^{13}C NMR and mass) analysis has been done for the catalyst and as well as for the synthesized compounds. Elemental analysis showed that the percentage of the nitrogen, hydrogen and carbon was found experimentally is approximately equivalent to the calculated values in all compounds. The doublets with coupling constants more or around 14.0Hz proved the chalcone structure, all the compounds gave the characteristic IR peaks around 16160-1610, 1720, 1760-1730 proved that the presence of C=C, C=O and lactam ring groups.



Scheme 1. TMS de-protection



Scheme 2. Schematic representation of the synthesis

Table 2. Recyclability of AAPTMS@MCM-41 tested for compound **5a**

Entry	Catalyst	Time (h)	Yield [a](%)
1	Fresh	2.0	90.0
2	2nd run	2.0	90.0
3	3rd run	2.0	88.0
4	4th run	2.0	85.0
5	5th run	2.0	75.0

[a] Isolated yields.

Table 3. Synthesis of carbapenem chalcone derivatives **1-12**

Entry	Product	R	Time (min)	Yield [a] (%)
1	CPC-1	H	8	90
2	CPC-2	4-NH_2	9	95
3	CPC-3	4-F	8	94
4	CPC-4	4-Cl	10	92
5	CPC-5	4-Nitro	8	93
6	CPC-6	4-OCH_3	7	88
7	CPC-7	4-Br	9	84
8	CPC-8	2,4-Di Cl	10	86
9	CPC-9	4-OH	8	90
10	CPC-10	2, 4-Diydroxy	9	95
11	CPC-11	2,4-Di Ome	10	94
12	CPC-12	4-N,N-Di Methyl	8	92

[a]Isolated yields.

At first the commercially obtained TMS protected carbapenem was deprotected as described in literature[24] (**Scheme.1**), later carbapenem chalcones derivatives **1-12 (Table 3)** were synthesized (**Scheme.2**) making use of the active SP^3methyl keto group hydrogens of carbapenem. Substituted aldehydes and carbapenem were taken into round bottom flask containing acetonitrile. The mixture was stirred for some time till the reactants gets well mixed, later catalyst AAPTMS@MCM-41 was added and the reaction was allowed to stir at RT, completion of the reaction was monitored by TLC. After the completion it was worked up by diluting with water and acidifying it with 1M HCl to bring the PH to 3, latter it was extracted using ethyl acetate and the catalyst was filtered off. The resulted solid was

purified using column chromatography to yield desired derivatives.

Table 4. In vitro cytotoxic activity of carbapenem chalcone derivatives (2-13) on two human cancer cell lines (IC_{50} µM/mL)

Entry	Compd	IC_{50} (µm/ML)	
		MCF-7	A-549
1	CP	7.25±0.008	5.76±0.006
2	CPC-1	4.78.±0.007	1.78±0.005
3	CPC-2	2.52±0.024	2.10±0.025
4	CPC-3	2.54±0.021	2.50±0.027
5	CPC-4	3.49±0.90	1.59±0.068
6	CPC-5	4.01±0.094	3.95±0.071
7	CPC-6	3.61±0.91	3.90±0.067
8	CPC-7	3.61±0.91	3.90±0.067
9	CPC-8	3.48±0.090	2.10±0.025
10	CPC-9	3.91±0.090	2.51±0.023
11	CPC-10	3.94±0.090	2.51±0.027
12	CPC-11	4.58±0.090	2.85±0.026
13	CPC-12	3.88±0.090	2.70±0.027
Standard	Cisplatin	3.55±0.007	1.56±0.005

Biology

In vitro anti-cancer activity of carbapenem chalcone derivatives

All synthesized carbapenem derivatives (1-12, Table 2) designated as CP were evaluated for their growth inhibitory activity against lung cancer (A-549) and breast cancer cell lines (MCF-7) using MTT assay (**Table 4**). The drug concentrations that inhibited 50% of the cell proliferation (IC_{50}) of the CP derivatives were calculated using reported methods [22], and are presented in Table 4, Among all derivatives tested CPC-2 (**Table 4 entry-3**) with amine substitution exhibited potent activity with IC_{50} value 2.52 µm/mL on MCF-7 breast cancer cell lines probably due to the ring activating nature of amine. CPC-4 (**Table 4 entry-5**) with chloro substitution exhibited better activity against A-549 lung cancer cell lines with IC_{50} value of 1.59 µm/mL.

Docking studies

Bio evaluation results were further conformed through docking studies, Anaplastic Lymphoma Kinase (ALK) is a tyrosine kinase enzyme that is encoded in humans by the ALK gene. This enzyme plays a very important role of aberrant signalling in cancer [23], and therefore, is a very promising target protein for the design of new anti-cancer agents. Similarly, high amino acids similarity of the active site of *β*-estrogen receptor (ER*β*) with its isoform, ER*α* [24] makes it an excellent target for the development of new anti-breast cancer drugs. In order to support our experimental activity profiles, the representative compounds were therefore docked into the active sites of both these proteins (ALK and ER*β*) using the Flexible docking algorithm in the DS [25], installed on the centre for high performance computing (CHPC), South Africa.

First, the efficiency and reproducibility of docking protocol was checked by re-docking the native ligand (crizotinib) in the active site of the ALK enzyme (pdb id: 2XP2). The root mean square deviation of the predicted conformation of the reference compound and its X-ray structure was around 1.2 Å (**Figure 6**), and validated the docking procedure.

The representative compounds (**Table 3**, CP, CPC-2 and CPC-4 and reference compound (cisplatin) were subsequently docked flexibly into the binding sites of the ALK and ERβ enzymes. The results obtained revealed that the most active compounds exhibited stronger binding affinities with the proteins. The computed binding energy of CPC-4 (-102.9 kcal/mol) was found to be significantly lower than CP (-17.0 kcal/mol), suggesting stronger interaction of the former with the ALK receptor. Similarly, the lower binding energy of CPC-2 (BE = -69.4 kcal/mol) relative to its structural analogue CP (BE = -39.2 kcal/mol) also favored its binding with the ERβ. The lowest BEs of cisplatin for ALK (109.0kcal/mol) supported its highest anti-cancer activity (IC_{50}=~1.56 µm/ML), whereas it's weaker interaction with ERβ (BE = -4.5 kcal/mol managed to explain it's lower potency compared to CPC-2.

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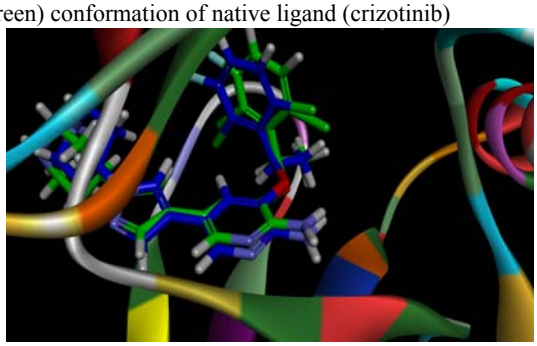
Figure 6. An overlay of the predicted (in blue) and crystallized (in green) conformation of native ligand (crizotinib)



In order to get a deeper understanding of the binding modes of compounds in the active sites of protein receptors, their complexes were visualized using the DS visualizer and are depicted in Figure 2-3. CP (**Figure 7a**) stabilizes its geometry in the binding site of ALK receptor *via* three hydrogen bonds; two concurrent hydrogen bonds between the carbonyl oxygen (ester group) and Met1199 (distance=1.97Å) and Leu1198 (distance=2.65Å), and a single hydrogen bond (distance=3.02 Å) between carbonyl group (lactam ring) and Gly1269. Compound CP-4, on the other hand, exhibited one hydrogen bond (distance=2.70Å) between carbonyl oxygen (lactam ring) and Arg394, and another hydrogen bond between aminic hydrogen (lactam ring) and Leu387 (distance=3.0Å), as depicted in **Figure 7b**.

In case of ERβ, CP (**Figure 8a**) exhibited two concurrent hydrogen bonds with Lys1150 (distances= 1.93 Å and 2.80 Å) through its carbonyl oxygen (lactam ring), and a single hydrogen bond (distance = 1.95Å) between hydroxyl group and Gly1269. The folded structure of CP was stabilized by an intramolecular hydrogen bond between lactam nitrogen and carbonyl oxygen of ester functionality. Additionally, the hydrophobic interaction between chlorophenyl ring and Leu1122 was also observed. CPC-2 interacted with the ERβ predominantly *via* hydrophobic interactions between its aromatic ring and Leu387, Ala350, Leu349, as shown in **Figure 8b**. Again, an intramolecular hydrogen bond accounting for its folded geometry was also present between its carbonyl oxygen (ester group) and hydroxyl group.

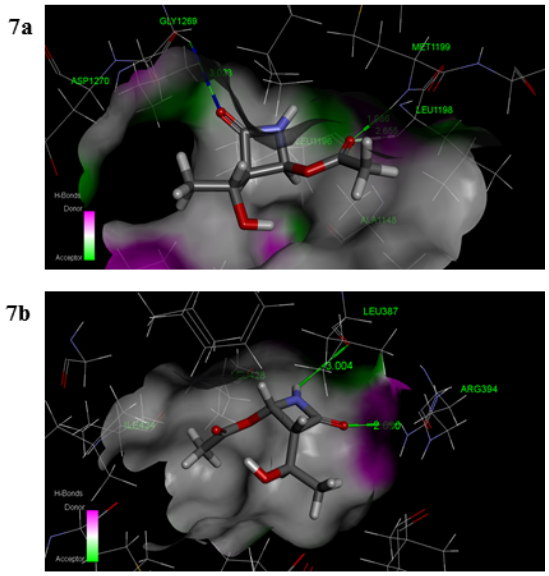


Figure 7. Docked conformation of CP (7a) and CPC-4 (7b) into the active site of the ALK enzyme. Compounds are shown in sticks format, whereas the amino acid residues of protein within 3Å are shown as surface whereas the remaining residues are depicted in lines format. Hydrogen bonds are presented in dotted lines.

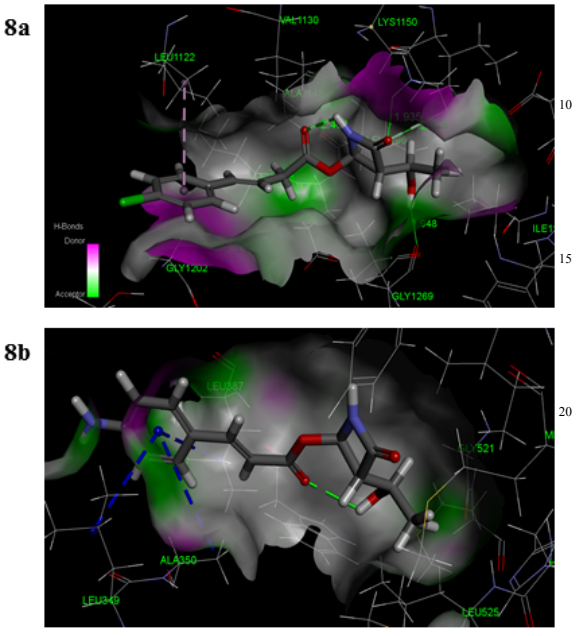


Figure 8. Docked conformation of CP (8a) and CPC-2 (8b) into the active site of the ERβ enzyme. Compounds are shown in sticks format, whereas the amino acid residues of protein within 3Å are shown as surface whereas remaining residues are depicted in lines format. Hydrogen bonds are presented as green dotted lines, while the hydrophobic interactions are depicted in dotted blue lines.

Experimental

TMS de-protection

Mixture of TMS protected carbapenem, n-hexane (7ml), silica sulpuric acid (0.05 g), and wet SiO_2 (0.2 g) was stirred at room temperature for 2 hrs. The reaction was monitored by TLC using 1:1 mixture of hexane and ethyl acetate. After completion of the reaction the mixture was filtered and the solid residue was washed with hexane. Upon evaporation gave pure alcohol.[24]

Preparation of diamine functionalized mesoporous silica

The mixture of cetyltrimethyl ammonium bromide (0.5 g), 2 M of NaOH (aq) (7 ml, 14 mmol), and H_2O (480 g) was heated at 80°C for 30 min at a pH of 12.4. To this clear solution, tetra ethyl ortho silicate (44.8 mmol) and AAPTMS (1.2ml) were added sequentially and rapidly. Following the addition, a white precipitation was observed after 3 min of stirring. The reaction temperature was maintained at 80°Cfor 2 h. The products were isolated by a hot filtration, washed with sufficient amount of water followed by methanol and dried under vacuum. For removal of surfactant, we are using acid extraction technique i.e., 1 gm of material were treated with mixture of ethanol (100ml) and con. HCL (1ml) at 80°C at 6 h. Then the mixtures were filtered and wash with ethanol, then dried at 80°C overnight. The sample was designated as AAPTMS@MCM-41**.**

Synthesis of Carbapenem chalcone derivatives

To a well-mixed solution of carbapenem (0.01 mol) and substituted aryl aldehydes (0.01 mol) in acetonitrile AAPTMS@MCM-41 catalyst was added and allowed it to stir foe few minutes, completion of the reaction was monitored by TLC. After the completion of the reaction it was worked up by diluting with water and acidifying it with 1M HCl to bring the PH to 3, latter it was extracted using ethyl acetate and the catalyst was filtered off. The resulted solid was purified using column chromatography to yield desired derivatives.

3-Phenyl-acrylic acid 3-(1-hydroxy-ethyl)-4-oxo-azetidin-2yl ester (Compound 1)

Yellow solid: mp 211-212°C; ^{1}H NMR (400 MHz, DMSO) δ = 1.84 (3H,s), 1.93 (1H,s, OH), 3.24 (q, 1H, *J* = 1.8 Hz), 4.61 (1H, d, *J* = 4.3 Hz), 5.62 (1H, d, *J* = 9.6), 7.28-7.33 (5H, m, Ar-CH), 7.84 (1H,d, *J* = 14.4), 7.95 (1H, d, *J* = 17.0) 8.5 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO): 23.31, 52.93, 62.04, 69.92, 125.59, 128.71, 130.22, 131.23, 139, 140.17, 175.59, 180.42; IR (cm^{-1}): 1660-1610 (C=C), 1720 (C=O), 1760-1730 (Lactam), 1720 (C=O), 3100-3000 (Ar C-H); MS (ESI), *m*/*z* = 262 (M+1, 100%); Anal. Calcd ($C_{14}H_{15}NO_4$): C 64.36, H 5.79, N 5.36%. Found: C 64.43, H 5.82, N 5.38%.

Docking Method

3D co-ordinates of the ELK (pdb id: 2XP2) and ERβ (pdb id: 1UOM) were obtained from their crystal structures uploaded in the protein data bank (http://www.rcsb.org). The native ligands and water molecules of both proteins were not considered in the calculations, and were removed using the DS visualizer. The protonated states of both proteins were determined at physiological pH using the Prepare Protein algorithm in DS. The proteins were minimized using the conjugate gradient algorithm to remove the bad contacts in the DS. Different conformational isomers of representative compounds (CP, CPC-2 and CPC-4) compounds were obtained using the Generate Conformations

module in DS. The lowest energy conformation of each RS was further geometrically optimized at DFT level using the combination of B3LYP functional and 6-31g [d,p] basis sets, in Gaussian 09.[26] A binding sphere covering all the active site residues was generated using the Define and Edit Binding Site module, and docking was subsequently performed using the Flexible docking algorithm[27] considering the default parameters. Of the total poses identified, the best docked pose was selected on the basis of its scoring function (-CDOCKER energy), and processed further for the binding energy calculations.

Conclusions

To conclude, a new class of carbapenem chalcone hybrid molecules were synthesized and evaluated for their anti-cancer activity along with docking studies. The most potent compound -2 **(entry-3)** with IC_{50} value of 2.52µM/mL represents the most better in vitro anti-cancer compound against MCF-7 breast cancer cell lines among the synthesized, and compound-4 **(entry-5)** displayed better anti-cancer activity against A-549 lung cancer cell line with IC_{50} value 1.59µM/mL. Furthermore compound-3 (entry-4) demonstrated good in vitro efficacies against both cell lines, additionally, docking of the representative compounds performed on the ELK and ERβenzymes revealed that most active compounds were stronger inhibitors of both enzymes, and showed a good correlation with their anti-cancer activity profiles.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

‡ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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