International Journal of Modern Agriculture, Volume 10, No.2, 2021
ISSN: 2305-7246

Synthesis, Characterization and Assessment of Carbendazim Based Mannich Analogues of Selected Clinical Agents

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Abstract

Benzimidazole and its derivatives especially Carbendazime (methyl 2 benzimidazole carbamate or MBC) represent an important pharmacophore having a wide variety of biological activities. This study aimed to explore the derivatives of a heterocyclic pharmacophore called Carbendazime. The present study includes the synthesis of N-mannich base derivatives of Carbendazime through a classical mannich reaction. Mannich reaction is a three components condensation reaction involving the condensation of a pharmacophore with an aldehyde to form an iminium ion, and then the resultant ion again condensed with a compound containing secondary amine. In this study, new mannich base derivatives of Carbendazime with secondary amine (Diclofenac, Aceclofenac, diphenhydramine and silver sulfadiazine) was synthesized via mannich reaction. Thin layer chromatography (TLC) and melting point (MP) determination techniques was used to ascertain the purity of the newly synthesized derivatives. For the structural elucidation of the newly synthesized compounds spectral techniques like FTIR, NMR, and mass spectroscopy etc was performed. Different anti-oxidant activities like DPPH, GST, GSH, SOD and Catalyze was performed for determining the pro oxidant potential of the synthesized derivatives. To study the toxicity Brine shrimp lethality assay and MTT assay was performed. Disc diffusion method was used for evaluating antifungal activity of the newly synthesized library. Among the synthesized compounds, compound DS and compound SS shows significant anti-oxidant potential via the above anti-oxidant assays. Furthermore the compounds do not show any significant toxicity against peritoneal macrophages through MTT assay. All the compounds exhibit potential anti-fungal activity. A library containing 4 derivatives of Carbendazime was synthesized showing increase pharmacological potential compared to the parent compounds, and are very interesting and potential candidates for future studies.

Key words: Carbendazim; Mannich Analogues; Clinical Agents

Introduction

Drug discovery is a vital and crucial process in the field of synthetic organic chemistry and medicinal chemistry. Drug discovery includes the synthesis, designing, characterization and pharmacological action of various compounds through screening libraries of smaller molecules, and the potency of such compounds to modify various pathways of biological systems in cell, tissues as well as in whole organisms without any specific target proteins (1). Many compound having different biological activities sharing some common structural features due to novelty and diversity in their complexity and activity (2).

The increasing number of algorithms that helps to evaluate and justify the interaction between the ligand and protein helps in docking studies(3). In Drug discovery docking is used for hit identification, lead optimization, active site analysis, structure based absorption, distribution, metabolism and excretion evaluation(4).

Mannich reaction is a carbon – carbon bond forming nucleophilic addition reaction in which a compound containing active (enolizable) hydrogen atom called substrate is reacted with an aldehyde (formaldehyde) and an amine either primary or secondary. Mannich reaction is preferred reaction. mannich reaction is a key step in various synthetic organic reaction that results in the preparation of wide variety of compounds including drugs, pharmaceuticals, and so forth(5). Mannich reaction results in compounds that containing beta-amino ketones which is called mannich bases (5). Mannich bases shows varied pharmacological activities including antibacterial, antifungal, anti-inflammatory, analgesic, antimalarial and anticonvulsant type of activities(6). There are many drugs (therapeutically active agents) that are chemically mannich bases in nature having amino alkyl chain in their structure and used clinically. for example antidiuretic agent like ethacrynic acid, antidepressant agent like fluoxetine , local anesthetic agents like cocaine, anticholinergic agents like atropine, procyclidine, biperidine, antiparkinsonian agents like trihexyphenidyl, proton pump inhibitors like ranitidine and so forth(5).

Benzimidazole derivatives including Carbendazime are a biologically active class of compound mainly used to control different disease of crops, and commonly called as fungicide. 1H benzimidazole is a well-known structure and an important pharmacophore in medicinal chemistry. It is a white to pale sandy fawn in color with a melting point of 172C°(7). Substituted benzimidazole having low toxicity with wide spectrum of biological activity is of great interest in the field of medicinal chemistry(8). Due to an extensive range of pharmacological activities different substituted benzimidazole are used as antiviral, antifungal, antimicrobial, antiprotozoal, anti-inflammatory, anticancer, antioxidant, anticoagulant, antidiabetic and antihypertensive activities. Another important property of benzimidazole and its various derivatives is that it can easily inhibit the biochemical pathway for ergosterol synthesis (9). The aim of our study was Synthesis, Characterization and Assessment of Carbendazim Based Mannich Analogues of Selected Clinical Agents.

Materials and methods

Animals and cultures

The different bacterial strains including Escherichia coli, pseudomonas aeruginosa, bacillus subtilis and staphylococcus aureus were used in our study. For bacterial strain culturing nutrient agar media was used and preserved at 37C°. The different fungal strains used in our study wer Aspergillus fumigate, Mucor species, Aspergillus flavus, Fusarium solani and Aspergillus nigar. Each species was cultured

on SDA (sabouraud dextrose agar) media and preserved at a temperature of 28 to 30 C°. Brine shrimp eggs were obtained from Ocean star Int; USA. Fluid from peritoneal cavity of rats was used for the extraction of macrophages. For macrophages extraction the peritoneal fluid was washed with phosphate buffer (pH 7.4), and then centrifuged at 13500 RPM for 10 minutes at 4C°. Carbon dioxide incubator was then used for their storage.

Preparation and purification of mannich bases

Mannich bases was prepared according to previous study (10). The purity of the synthesized compounds was checked by TLC. Merck TLC silica gel60 was used for this purpose.

Sample characterization

The sample characterization was done through solubility profile, Melting point determination, thin layer chromatography and Spectroscopic techniques.

In Silico Studies

An Insilico study of the newly synthesized library was done by using various date bases and different online web tools. Different software and data bases were used for Insilico studies of my novel library that includes Swiss target prediction, Molecular docking, Swiss ADME and Molsoft.

Biological evaluation

The biological activities of newly synthesized chemical library were evaluated through different invitro assays. These assay include Anti-oxidant assays, GST activity (assay), GSH assay, Cytotoxicity Assays (Brine shrimp lethality assay and MTT Assay), Nitric oxide assay, Anti-bacterial assay and Anti-Fungal Assay.

Data analysis

Data was entered and analyzed by using SPSS version 23. Mean and standard deviation were documented for continuous variables while for categorical data, frequency and percentages were reported. All the data was presented in the form of tables and figures.

Results

Expected (IUPAC) Names of the Newly Synthesized Compounds

Table 1: shows the expected chemical name (expected IUPAC name) of the newly synthesized compounds)

S/No.	Product code	Chemical name (expected IUPAC name)
01	DS	2-(2-((2,6-dichlorophenyl)((2-((methoxycarbonyl)amino)-1H-benzo[d]imidazol-1-yl)methyl)amino)phenyl)acetic acid
02	AC	2-(2-(2-((2,6-dichlorophenyl)((2-((2-methoxy-2-oxoethyl)amino)-1H benzo[d]imidazol-1-yl)methyl)amino)phenyl)acetoxy)acetic acid
03	DH	methyl (1-(((2-(benzhydryloxy)ethyl)(methyl)amino)methyl)-1H-benzo[d]imidazol-2-yl)carbamate

Solubility Studies

Table 2: Solubility profile of newly synthesized library along their code and serial number

s/no.	Sample code	Solubility profile
01	DS	Soluble in DMSO, ethanol, DCM and chloroform Insoluble in water, n hexane
02	AC	Soluble in Ethanol, DMSO and ethyl acetate Insoluble in chloroform and water
03	DH	Soluble in DMSO and ethanol Sparingly soluble in chloroform
04	SS	Soluble in DMSO, ethanol and n hexane Insoluble in chloroform and DCM

In Silico Studies

The best docked poses for each compounds were selected which revealed that, inhibitors are very well accommodated in the active site. The compound coded as DS and DH showed good interaction with cox-1 with binding energy scores of -3.47 and -9.66 respectively. While in case of cox-2 all the three compounds showed interactions with binding score of -7.18, -6.14 and -7.31 respectively. The binding interactions involved were hydrogen bonding, pi-pi T-shaped, pi-alkyl, pi-anion, pi-pi stacked, pi-sigma and also halogen interactions. The Amino acids involved in binding were tyr 385, Ile 523, Ala 527, ser 530, leu 352, val 349, tyr 355, leu 531, leu 359, val 116, Arg 120, ser 353 in case of cox-1 and in cox-2 were val 116, Arg 120, Tyr 355, Leu 352, Glu 524, Val 523, Val 349, Ala 527, Ile 345, Ser 530, Leu 531.

Docking pose of meloxicam with COX-1 and COX-2

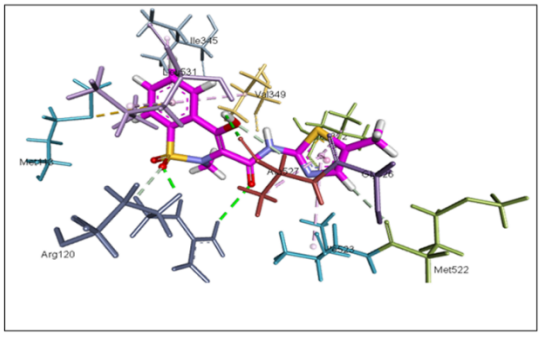


Fig.1.
Predicted docking pose of meloxicam in active site Cox-1 enzyme

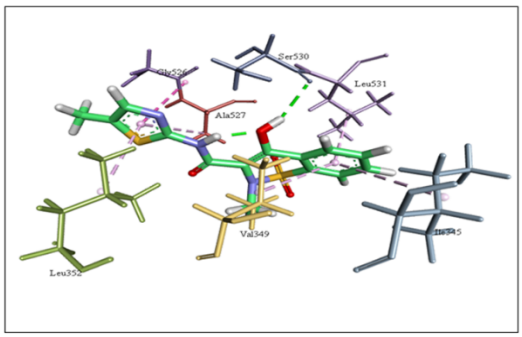
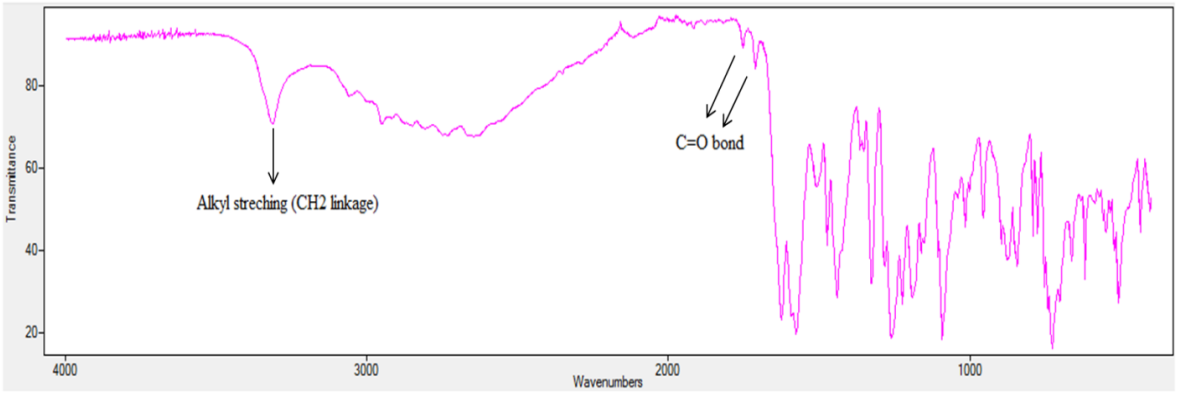


Fig.2.
Predicted docking pose of meloxicam in active site Cox-2 enzyme

FTIR (Fourier transform infra-red) spectroscopy

During mannich reaction heterocyclic ring remains intact giving peak above 2700cm^{-1}. The presence of peak at 1650cm^{-1} indicates the presence of carbonyl (C=O) linkage that gives confirmation about many products. Basically the presence of C=C and C=N bonds gives peaks in the range of 1200cm^{-1} to 2350cm^{-1}, indicating the completion of mannich reaction. Similarly the presence of strong aromatic alkyl stretching in case of compound coded as AC, DS and DH gives us peak at 3350cm^{-1}



The figure 3: FTIR spectra of the synthesized compound coded as AC.

Anti-oxidant assay

All the newly synthesized compounds showed significant free radical scavenging activity. As from the figure it is clear that the percent scavenging activity of the newly synthesized compounds increases by increasing their molar strength from 1 µm to 100 µm.

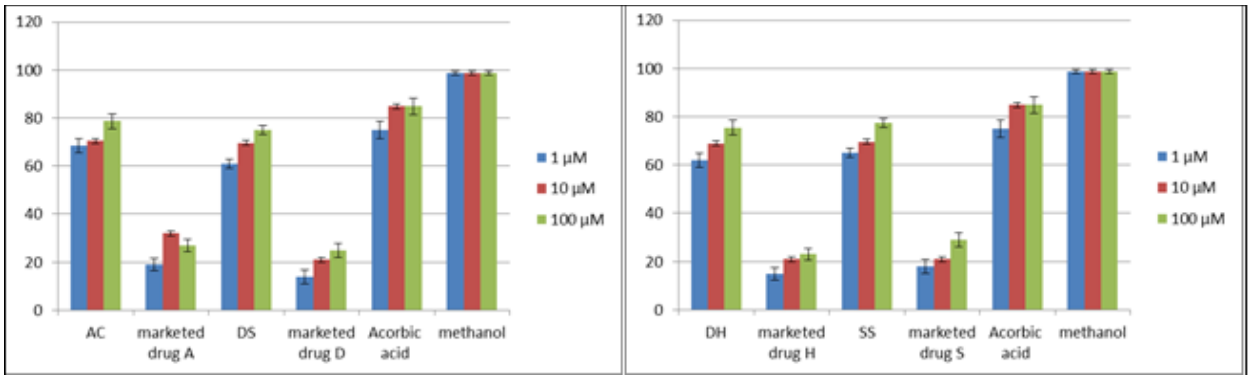


Figure 4: Graph showing the results of percent (%) scavenging activity of already marketed drugs and the newly synthesized compound.

Glutathione – S – Transferase (GST) assay

Maximum GST production was calculated in case of compound coded as DS. Generally all the newly synthesized compounds showed significant GST production.

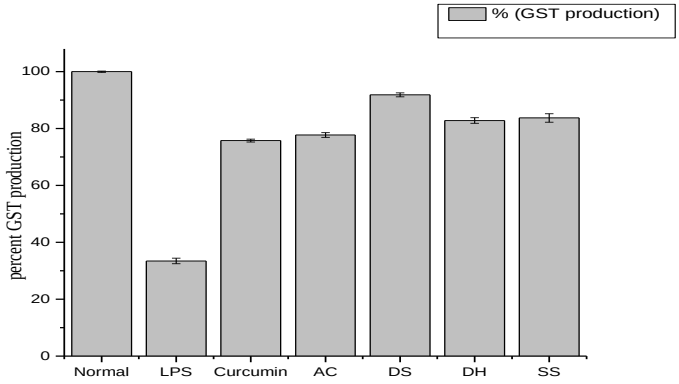


Figure 5: Graph representing the % GST production by the test samples (newly synthesized compounds) as well as by the positive, negative and normal control group.

Glutathione (GSH) assay

All the newly synthesized compounds show good antioxidant potential by stimulating the production of GSH.

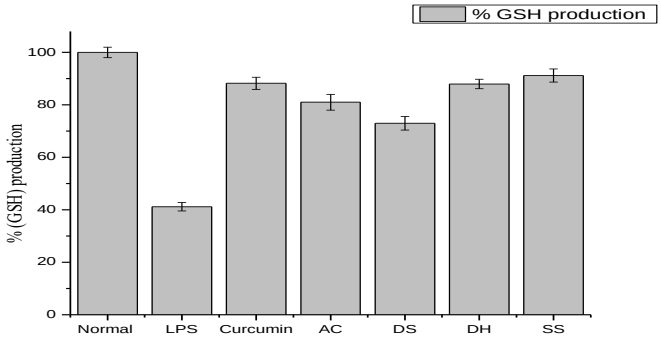


Figure 6: Plot representing the % GSH production by the test samples as well as by the positive, negative and normal control group

SOD assay

All the compounds have good high SOD production except for the compound coded DS, So all the compounds possess good anti-oxidant properties.

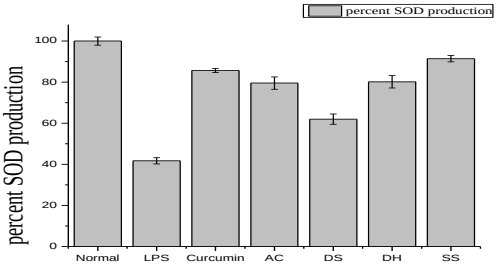


Figure 7: Graph showing % SOD production by different test samples as well as by normal control, LPS and curcumin

MTT cell viability assay

Among the newly synthesized compounds, the compound coded as DC shows maximum % cell viability at molar concentration of 1 µm. compound coded as SS and DH also shows significant %cell viability at molar concentration of 1 µm. Compound AC shows moderate %cell viability at the same concentration.

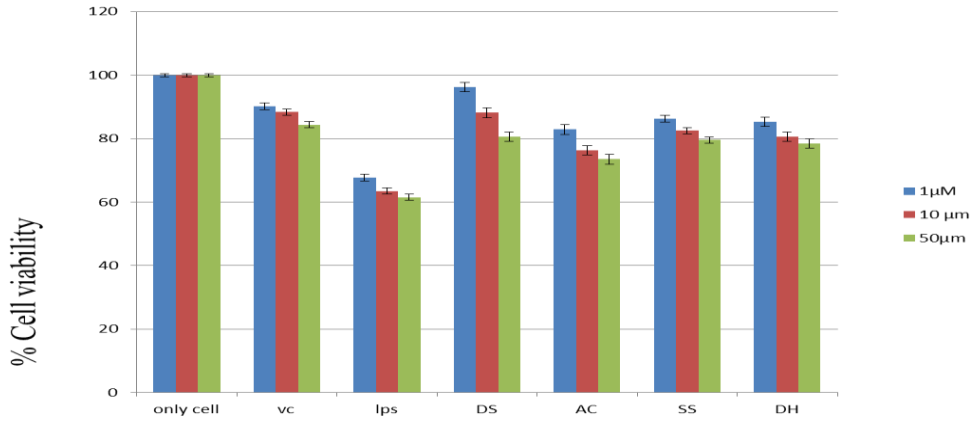


Figure 8: Shows the % cell viability of the newly synthesized compounds as well as vehicle control, only cell and LPS

Antifungal assay

All the newly synthesized compounds were only active against fumigatus fungal strain but not active against any other five fungal strains.

Table 3: Plot the zone of inhibitions of different newly synthesized compounds against different fungal strains

Sample code	Diameter of Zone of inhibition (mm)									
	A. fumigatus (ZOI)	MIC 26µg/ml	F. solani (ZOI)	MIC 26µg/ml	Mucor (ZOI)	MIC 26µg/ml	A. flavus (ZOI)	MIC 26µg/ml	A. niger (ZOI)	MIC 26µg/ml
AC	9mm	--	-ive	-	-ive	-	-ive	-	-ive	-
DS	11mm	--	-ive	-	-ive	-	-ive	-	-Ive	-
DH	10mm	--	-ive	-	-ive	-	-ive	-	-ive	-
SS	7mm	--	-ive	-	-ive	-	-ive	-	--ive	-

Anti-bacterial assay

All the compounds don't show any significant antibacterial property.

Cytotoxicity assay

Among all many newly synthesized compounds only compound coded as AC and DS showed significant cytotoxicity > 50% at a concentration of 400µg/ml.

Table 4: Table representing % mortality of different tested samples

Samples code	Percent (%) mortality (Conc. µg/ml)				LD_{50} µg/ml
	400	200	100	50	
Positive control	80±1.3	60±1.5	40±1.25	20±1.26	409.32
AC	55±2.166	43±2.1	23±2.1	21±1.29	286.34
DS	61±1.9	40±1.8	23±1.23	20±1.21	310.33
DH	38±1.16	30±1.26	30±1.15	18±1.24	-
SS	41±1.26	31±1.2	31±1.4	25±2.11	-

Nitric oxide assay

At different concentrations of the test samples NO production was measured. The most potent compound was DS in inhibiting the NO production. All other compounds inhibit NO production significantly but compound coded as DS and AC were the most potent compounds showing good anti-inflammatory properties.

Table 5: Shows the result of NO assay of different test samples along with T test in tabular form

Sample code	% NO							
	100µM	T test	50 µM	T test	10 µM	T test	1 µM	T test
DS	3.2	$1.2*10^{-6}$	5.9	$1.3*10^{-6}$	10.9	$2.3*10^{-4}$	25.2	$3.3*10^{-2}$
AC	3.5	$1.4*10^{-6}$	4.9	$1.6*10^{-5}$	12.4	$2.5*10^{-4}$	24.5	$1.2*10^{-2}$
DH	8.1	$1.9*10^{-5}$	12.6	$2.8*10^{-4}$	15.9	3.510^{-3}	29	2.110^{-1}
SS	10.11	$2.4*10^{-5}$	14.5	$2.6*10^{-4}$	18.5	$4.5*10^{-3}$	32.4	$3.6*10^{-1}$

Discussion

In present study by applying simple and reliable synthetic multi-component mannich reactions (MCMR), we have synthesized four different aminomethylated derivatives of the pharmacophore (Carbendazime).

The standard protocol for performing mannich reaction described by (10) with little modification was followed for the synthesis of my novel compounds. The presence of two aromatic rings in 2 methyl

benzimidazole structure enhances the binding affinity with different enzymes (receptors) through hydrogen bonding and hydrophobic interactions(11).

Due to excessive use of non-specific antibiotics in various infectious conditions, bacterial resistance in human body increases day by day. The newly synthesized compound efficacies towards different bacterial and fungal strains are because of multiple reasons, not due to innate antifungal and antibacterial nature of the pharmacophore (Carbendazime). The nature and structure of the attacking amine also affect the overall activities of the newly synthesized compounds. The antimicrobial properties of newly synthesized mannich base derivatives of Carbendazim is also due to the presence of electron withdrawing and electron donating groups present in their structures.

All the newly synthesized compounds showed some antifungal activity against fumigatus fungal strains. This antifungal property is mainly due to the pharmacophore (Carbendazim), and also due to the presence of electron withdrawing groups in the main structure(12). The presence of electronegative groups (electron withdrawing groups) and the attached substituents with them through pi-bond was also responsible for antifungal properties(13). Besides this the hydrophobic domains (present in the constrained ring system) as well as hydrophilic nature of the carboxylic acid chains (unsaturated side chains) also enhance the antifungal activities of newly synthesized compounds. This was supported by the literature review on antimicrobial behavioral studies(14).

. All the newly synthesized compounds of my synthetic library showed good antioxidant potential. This property of new synthesized (15) compound was checked and confirmed by different in vitro antioxidant assays and the results were shown in the result section. In free radicle scavenging assay (DPPH assay) there was a positive correlation between the scavenging activity and the concentration all the synthetic compounds of our library. The scavenging activity of compounded coded AC, DS and SS was very high (upto 80%) and that of compounded coded as DH was moderate (upto 65%) compared to the standard drug. From the literature review a similar correlation was also seen in (16). The literature review on the antioxidants showed that the length of alkyl chain has a positive relation with the antioxidant properties of the compounds. From the SAR study of the compounds it is clear that by increasing the length of side alkyl chain antioxidant potential is greatly enhanced. The presence of electron rich aromatic system is responsible for the redox property of our compounds. This nucleophilic aromatic ring system make the amine and imidazole ring more prone to electrophilic attack and can easily donate electrons supported by(17). The antioxidant potential of these compounds was supported by the literature study(18).

The two main components of defense system responsible for detoxification and neutralization of harmful reactive species and electrophiles were GSH and GST. Both acute and chronic damaging effect of ROS and RNS was reversed by this both components of defense system. Compounds having electron withdrawing groups like compounds coded as DS and AC causes increase production of GSH and GST. It means that compounds having high GST and GSH production have more ability to conjugate more free radicles and as a results they reduces the oxidative stress. Actually aromatic rings having substitutions at position number 2 or 3 causes increase production of GST (19).

Compounds coded as DS and SS shows greater % cell viability at 1µm and by increasing molar concentration % cell viability decreases signicantly, showing that % cell viability is inversely related to concentration of the newly synthesized compounds.

In the present study the anti-inflammatory activities of the newly synthesized compounds was evaluated by inhibiting the NO production which was caused by LPS in the macrophages. All the compounds

showed good anti-inflammatory potentials by decreasing NO production in the LPS infected macrophages. This anti-inflammatory potential of all the synthetic compounds was also supported by literature review (20). The anti-inflammatory potentials of compound coded as DS and AC are due halogens groups that are electron withdrawing groups, and supported by (15).An Insilico study of the newly synthesized compounds was done so as to confirm the binding site as well as binding affinities with the target proteins. This Insilico studies was done by using online data bases and online web tools. For computational studies of newly synthesized compounds various online free software were used including Molsoft, Swiss ADME, docking and Swiss target prediction etc. Drug likeliness potential of all the newly synthesized compounds was determined so as to find those that are in compliance with that of acceptable drug profile (21). All the newly synthesized compounds showed drug likeliness by obeying all the five rules of Lipinski's. One of the compound coded as DS violated only one rule having molecular weight greater than 500. The knowledge about the BBB crossing of drugs and GIT absorption profile was obtained from the value of W log P/ TPSA (topological surface area) that were calculated by Swiss ADME software. The interaction between a new ligand molecule and a target is predicted by software called molecular target prediction and this is an important and crucial step in the identification of new drug. Computational studies including molecular target prediction provides us important information about the basic biological mechanism of drug action (22). The modulation of biological pathways by various biological and clinical agents in the animal body to obtained the desired pharmacological action also predicted by molecular target prediction (23). In docking studies first of all a suitable target was selected from protein data base. After target selection that was COX (Cyclo-oxygenase enzyme) all the newly synthesized compounds was docking with specified target. As the heterocyclic pharmacophore (Carbendazim) already possess binding affinity for that target. On the basis of this observation it is concluded that the binding affinity of all of newly synthetic chemical library is mainly because of the heterocyclic ring system of pharmacophore which was not disrupted during chemical reaction.

Conclusions

After conducting a series of biochemical and biological assays, the results of all these assay were compiled which shows that newly synthesized mannich bases derivative of Carbendazime represents an important class of compounds possessing a wide variety of biological activities. The class of newly synthesized compounds showed good biocompatibility. Furthermore the results calculated also give us the idea that all the newly synthesized compounds showed good anti-inflammatory activities. The result of GST, GSH, SOD and catalase assays clearly indicates that all the newly synthesized mannich base derivatives have good antioxidant potential. One of the derivatives containing sulfadiazine as an amine, possess some antibacterial potential. Mannich base derivative containing electron withdrawing groups also possess antifungal activities against fumigatus strains of fungi. Docking studies gives us information about the binding proteins. The result of docking clearly indicates that the newly synthesized compounds have high binding affinity with both COX-1 and COX-2 enzymes, but mostly with COX-1. From the cytotoxicity assay, all the compounds have concentration dependent cytotoxicity. At last from the result of all the assays it is concluded that the newly synthetic chemical library have good and prominent analgesic anti-inflammatory potential and are suitable agents for multi-purpose drug development.

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