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Synthesis and Evaluation of 2-Mercapto benzimidazole Mannich Base Derivatives as a Carbonic anhydrase II inhibitors

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ABSTRACT

Background: The synthesized compounds showed moderate CA II inhibitory activity (IC50 = 182.20-222.21 uM), while compound 2f demonstrate the highest antioxidant potential (IC50=5.11uM). therefore, 2-mwercaptobenzimidzole-based Mannich derivatives may serve as attractive leads for the development of novel non classical CA II inhibitors possessing antioxidant activity. The 2-Mercaptobenzimidazole and Carbonic Anhydrase Inhibitors (CAIs) Mannich bases were also found to have antioxidant activity. **Method:** The target compounds were prepared using reflux condensation method and their structure was confirmed by FTIR and proton NMR (^{1}H NMR) spectroscopy. The biological potential was evaluated through antioxidant activity using the DPPH assay, inhibitory activity against CA II by in vitro study and molecular docking study on the protein PDB: 1A42. **Results:** Docking analyses reveal that among the synthesized compounds 2g, 2e, 2h, and 2f exhibit the highest calculated binding affinities, whereas the compounds exhibit moderate CA II inhibitory activity (IC50=182.20-222.21 uM) and compound 2f demonstrate the highest antioxidant potential (IC50=5.11uM). **Conclusion**: Therefore, 2-mwercaptobenzimidzole-based Mannich derivatives may serve as attractive leads for the development of novel non classical CA II inhibitors possessing antioxidant activity.

Keywords: 2-Mercapto benzimidazole, Mannich derivatives, Carbonic anhydrase II inhibitors, Synthesis

INTRODUCTION

Carbonic anhydrases (CAs) are enzymes that catalyze the reversible reaction between CO_2 and water: $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$, and are widely distributed in the tissues of higher organisms. There are at least 16 human isoforms (CA I–XV) known, which all fall into the αclass and have three histidine residues which chelate the catalytic Zn(II) ion. Isoforms have varying tissue distribution and subcellular localization, such as cytosolic, mitochondrial, membrane-bound and secreted [1, 2].

All CAs involve in pH homeostasis, CO_2 transport, electrolyte secretion, gluconeogenesis and calcification. They are involved in the dysregulation of glaucoma, epilepsy, renal tubular acidosis, osteoporosis, neuropathic pain and tumorigenesis. CA IX and CA XII are validated targets for cancer therapy in hypoxic tumors [3].

Classical CA inhibitors (CAIs) are sulfonamides (RSO_2NH_2) that bind the catalytic zinc and replace the zinc-bound water molecule in the catalytic site. Though useful clinically, they are non-selective inhibitors of several isoforms, leading to side effects like

fatigue and nausea. Alternative binding modes and greater isoform selectivity exist for non-classical CAIs such as phenols, polyamines and coumarins [4, 5, 6]. Several clinically used antiepileptics (topiramate, zonisamide) also potently inhibit CNS CA isoforms [7].

2-Mercaptobenzimidazole ($C_7H_6N_2S$) possesses broad pharmacological activity including antibacterial, antioxidant, and anticarcinogenic properties. It is characterized by a thioamide (N–C=S) group and is in the state of thiol–thione tautomerism. Mannich bases are pharmacophores, which have a broad range of biological activities, established bases. Thus, a rational approach to the development of non-sulfonamide CAIs involved the hybridization of the 2-mercaptobenzimidazole with a Mannich base moiety. In this study, nine new derivatives (2a–i) of CA II inhibitor were synthesized, characterized, evaluated for its antioxidant activity, and performed molecular docking with the enzyme CA II (PDB ID: 1A42).

METHODOLOGY

2.1. Chemicals

Chemicals were purchased from Sigma-Aldrich, Alfa Aesar, DAEJUNG, Merck and Fluka. The most important reagents were 2-mercaptobenzimidazole, benzyl chloride, diethyl amine, morpholine, p-anisidine, formaldehyde, acetaldehyde, benzaldehyde and salicylaldehyde. Solvents used (ethanol, methanol, ethyl acetate, and petroleum ether) were all obtained from either LAB-SCAN or Fisher Scientific.2.2 Synthesis

Step 1 – 2-(Benzylsulfanyl)-1H-benzimidazole (1): 2-Mercaptobenzimidazole (3.3 mmol) was added to dry ethanol (10 mL) and benzyl chloride (1.2 equiv.) was added and the mixture was heated under reflux for 2 h. The product was filtered, washed with cold water and recrystallized from methanol. Yield: 89%; m.p. 165°C [13]. Step 2 – Mannich base derivatives 2(a–i): Compound 1 and the respective aldehyde (equimolar, 0.01 mol) in dry ethanol (10 mL) were refluxed for 1 h. The secondary amine was added and refluxing continued for 8–10 h under mild HCl catalysis. Products were filtered and recrystallized from ethanol. Reaction progress was monitored by TLC (ethyl acetate:petroleum ether, 1:2) [14].

All compounds were characterized by ALPHA FTIR (ATR mode) and ^{1}H NMR (Bruker AM-300, 300 MHz, DMSO-d_6). Melting points were measured on a Stuart apparatus. Purity was confirmed by TLC (Silica gel 60 F254).

2.2 DPPH Antioxidant Assay

DPPH (9.2 mg/100 mL methanol) and test compounds (4 mg/mL DMSO) were added to 96-well plates (10 µL sample + 190 µL DPPH per well). After incubation at 37°C for 60 min in the dark, absorbance was read at 517 nm. Ascorbic acid served as the positive control. % Scavenging = (1 − Abs_{test} / $Abs_{h\square ntol}$) × 100. Assays were run in duplicate [15].

2.3 Carbonic Anhydrase II Inhibition Assay

Assays were conducted in 96-well plates at 25°C in 20 mM HEPES-Tris buffer (pH 7.4). The test compound (20 µL) in DMSO, bovine erythrocyte CA II (20 µL of 0.1mg/mL) and buffer (140 µL) were added to each well. The reaction was started with 20 µL of 4-NPA substrate (0.7 mM) and was monitored 30 min at 400 nm, with acetazolamide as standard. % Inhibition = 100 − (OD_{tes-t} / $OD_{h--hntoll}$) × 100. Triplicate measurements were made. To make any sense of the data, statistical analysis such as the mean, SEM, and significance testing should be reported [16].

2.4 Molecular Docking

The crystal structure of the CA II (PDB ID: 1A42) was downloaded from the RCSB Protein Data Bank. To prepare protein, the water molecules and any co-crystallized ligands were removed by using Discovery Studio Visualizer (v17.2) [17]. The molecules of the ligand were first drawn in ChemSketch and then optimized in the 3D geometry. The optimized structures were then converted to PDBQT format using the Argus Lab and Auto Dock Tools (v1.5.6) programs.

We carried out docking analysis with Auto Dock Vina (v1.1) [18] and the docking poses of lowest binding energies were chosen for further analysis. Discovery Studio was used to analyze the interactions between the protein and ligands, and to create 2D and 3D interaction diagrams. Additionally, a set of chemo-informatics properties of the compounds was performed using

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Molinspiration and ChemSketch [19, 20]. It is highlighted that the reporting of the dimensions and coordinates of the grid, exhaustiveness parameters and docking validation by re-docking of the co-crystallized ligand with RMSD calculation should be a part of any revision of this work to enable full reproducibility and methodological transparency.

RESULTS

3.1 Chemo-informatics

The synthesized compounds were found to be drug-like by performing a drug-likeness assessment, which met Lipinski's rule of five with appropriate log values, number of hydrogen bond donors and acceptors, and PSA values. This suggests that the compounds will probably be effective if swallowed. You can see the details in Table 1. It's good because the compounds were ideal for oral bioavailability. The molecular weight, logP and other such things were all within limits, that is good for the compounds and their oral bioavailability.

Table 1: Chemo-informatics properties of synthesized compounds

Compound	HBA	HBD	LogP	PSA (Å^2)	Molar Refractivity	Density (g/cm^3)	Lipinski
2a	3	0	4.78	21.06	100.87 ± 0.5	1.11 ± 0.1	Yes
2b	3	0	5.14	21.06	105.29 ± 0.5	1.10 ± 0.1	Yes
2c	3	0	6.36	21.06	125.97 ± 0.5	1.11 ± 0.1	Yes
2d	4	1	6.30	41.29	126.82 ± 0.5	1.16 ± 0.1	Yes
2e	4	1	5.90	39.09	117.24 ± 0.5	1.18 ± 0.1	Yes
2f	4	0	4.24	30.30	104.50 ± 0.5	1.23 ± 0.1	Yes
2g	4	0	5.46	30.30	125.18 ± 0.5	1.22 ± 0.1	Yes
2h	5	1	5.39	50.53	126.03 ± 0.5	1.28 ± 0.1	Yes
2i	4	0	3.87	30.30	100.08 ± 0.5	1.24 ± 0.1	Yes

3.2 Characterization

Structures were confirmed by FTIR and ^{1}H NMR. FTIR showed characteristic absorptions: C=C (1515–1589 cm^{-1}), C=N (1600–1640 cm^{-1}), C–H (2900–2968 cm^{-1}), N–H (3310–3359 cm^{-1}), and C–S (658–692 cm^{-1}) in all derivativesIn the NMR test the hydrogen atoms that are near the sulfur show up as a peak at around 4.46. The hydrogen atoms that are part of the ring show up as many peaks between 6.18 and 8.01. The hydrogen atom of the Mannich base part shows up between 2.40 and 4.46. The number of peaks, for the Mannich base hydrogen atom depends on the kind of aldehyde linker that is used in the Mannich base. The NMR test is looking at the methylene proton and the aromatic protons and the methine proton of the Mannich base moiety.

3.3 Antioxidant Activity (DPPH Assay)

The antioxidant activity of something was measured. This was done by seeing how well it could stop DPPH radicals. Ascorbic acid was used as a standard to compare with. The IC_{50} value of ascorbic acid is 7.48 µM. This means that the antioxidant activity of the thing being tested can be compared to ascorbic acid. Compound 2f showed the highest activity (IC_{50} 5.11 µM), followed by 2e (22.18 µM), 2g (31.48 µM), and 2c (32.27 µM). All compounds exhibited >50% radical scavenging potential (Table 2).

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Table 2: DPPH Assay results and IC_{50} values

Compound	% Scavenging	IC50 µg/ml	IC50 µM ± SEM%
2a	64.47	12.12	37.24±0.033
2b	57.30	26.56	78.23±0.035
2c	67.32	12.96	32.27±0.042
2d	64.69	21.76	52.11±0.048
2e	68.80	8.64	22.18±0.040
2f	85.71	1.82	5.11±0.042
2g	66.34	13.08	31.48±0.044
2h	67.82	14.52	33.65±0.050
2i	66.01	11.36	33.46±0.033
Ascorbic acid (std)	—	—	.48

3.4 Carbonic Anhydrase II Inhibition

The compounds 2e, 2f 2g and 2h were tested in a lab, against bovine erythrocyte CA II.The compounds 2e, 2f 2g and 2h all showed that they could stop the activity of bovine erythrocyte CA II. This happened when the compounds 2e 2f 2g and 2h were used in micromolar range.

Compound 2h (IC_{50} 182.20 µM, 57.43% inhibition) and 2f (IC_{50} 187.65 µM, 62.37%) were the most active within the series. Acetazolamide, which is what we usually use to compare with stopped the process by 70.40 percent with an IC_{50} of 388.49 µM when we did the assay the way (Table 3). We need to remember that the IC_{50} value we found for Acetazolamide in this test is higher than what people say it is. This difference is probably because of the conditions we used for the assay, like how much substrate we used, where the enzyme came from and what kind of buffer system we used. So when we look at these results we have to think about the setup we used for the assay. Differences in experimental condition require caution when making directs comparison with earlier report.

Table 3. Inhibitory activity of synthesized compounds against Carbonic Anhydrase II (CAII).

Compound	% Inhibition	IC50 µg/ml	IC50 µM ± SEM%
2e	59.94	79.55	204.23±0.200
2f	62.37	67.10	187.65±0.207
2g	55.97	92.34	222.21±0.286
2h	57.43	78.63	182.20±0.083
Acetazolamide (std)	70.40	86.34	388.49±0.475

3.5 Molecular Docking

Binding affinities were ranked: 2g > 2e > 2h > 2f > 2i > 2d > 2c > 2b > 2a > acetazolamide Compounds 2g and 2e exhibited the strongest binding affinities, with value of -8.3 and -8.1 Kcal/mol, respectively, compared to -5.8 kcal/mol observed for acetazolamide.

Key active-site residues involved included HIS 91, VAL 131, VAL 139, VAL 118, LEU 194, PHE 127, PRO 198, GLN 89, and HIE 116 (Table 4).

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Table 4: Molecular docking results against CA II (PDB ID: 1A42)

Compound	Amino Acids Involved	Binding Energy (kcal/mol)
2a	HIS 91, VAL 131, ALA 62, THR 196, VAL 139, VAL 118, LEU 194, ASN 59, PHE 127	-6.8
2b	HIS 91, VAL 131, THR 196, VAL 139, VAL 118, LEU 194, PHE 127, PRO 198	-6.9
2c	HIS 91, VAL 131, THR 196, VAL 139, VAL 118, LEU 194, PHE 127, PRO 198, ILE 88, PRO 197, GLN 89, HIE 116	-7.4
2d	HIS 91, VAL 131, VAL 139, VAL 118, LEU 194, PHE 127, PRO 198, ILE 88, PRO 197, GLN 89, HIE 116	-7.5
2e	HIS 91, VAL 131, VAL 139, VAL 118, LEU 194, PHE 127, PRO 198, GLN 89	-8.1
2f	HIS 91, VAL 131, VAL 139, VAL 118, PHE 127, PRO 198, HIE 116, THR 196	-7.6
Compound	Amino Acids Involved	Binding Energy (kcal/mol)
2g	HIS 91, HIS 61, VAL 139, VAL 118, LEU 194, PHE 127, PRO 198, GLN 89, HIE 116	-8.3
2h	HIS 91, VAL 131, VAL 139, VAL 118, PHE 127, PRO 198, ILE 88, PRO 197, GLN 89, HIE 116	-7.9
2i	HIS 91, VAL 139, VAL 118, LEU 194, PHE 127, GLN 89, ASN 64	-7.6
Acetazolamide	ASN 59, THR 196, LEU 194, THR 195, GLN 89, ASN 64	-5.8

DISCUSSION

Benzimidazole derivatives have been reported to exhibited antimicrobial, antimicrobial and enzymes activities. Mannich base hybridization is commonly employed strategy to enhance the biological potency of heterocyclic compounds, and this approach was used in the synthesis of the present series. The structure of the synthesized compounds was confirmed through FTIR and NMR spectroscopic analysis. Benzimidazole derivatives have been reported to exhibited antimicrobial, antimicrobial and enzymes activities. Mannich base hybridization is commonly employed strategy to enhance the biological potency of heterocyclic compounds, and this approach was used in the synthesis of the present series. The structure of the synthesized compounds was confirmed through FTIR and NMR spectroscopic analysis.

The DPPH radical scavenging assay revealed that compound 2f exhibits the highest radical scavenging activity (IC_{50} = 5.11 µM), which is less than that of asscorbic acid (IC_{50} = 7.48 µM) under the same conditions indicating a significant free radical scavenging property [15]. This could be due to the ability of the morpholine N to serve as an electron donating group and thus be able to stabilize radical intermediates. The antioxidant potential of benzimidazole derivatives has been reported before and is thought to be due to their potential to donate electrons or hydrogen atoms to the ROS [8]. The scavenging activity values of compound 2e and 2g were also found to be appreciable (IC_{50} values 22.18 and 31.48 µM respectively) while compound 2b was weak (78.23 µM), probably because the electron withdrawing effect of the substituents on the imidazole ring decreases the electron density at certain positions. The property of antioxidant activity is not a primary pharmacological target of this series and it is reported as a secondary finding, which is in line with the pharmacological profile reported for 2-mercaptobenzimidazole scaffolds [8, 14]. The data were run in duplicate and this reduces the statistical robustness of the data and so replication in triplicate with appropriate statistics is recommended to gain confidence in the reported IC_{50} values [15]. The four compounds that were tested all showed some ability to stop the anhydrase II enzyme from working. This happened when the compounds were used in amounts of 182 to 222 micromoles per liter. For comparison a reference compound called acetazolamide was also. It took 388.49 micromoles per liter to get the same effect. This is a lot higher than what other people have found in their research.

The reason for this difference is probably because of the way the test was done. The test used a version of the enzyme that comes from cows than the version that humans have. The test also used a substance and conditions that might be different from what other people used. It is worth noting that the cow version of the enzyme is very similar to the version with about 79 percent of the parts being the same. However, there might be some differences that affect how well the compounds work.

Thus, it is necessary to take care with direct comparisons between our results and those of other studies. The synthesized compounds showed moderate activity indicating that further modifications to the structures are likely to improve the activity.

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The interesting feature of these compounds is that they do not include the sulphonamide group, which has been regarded as essential to activity for numerous other structurally related inhibitors. Direct comparisons with previous reports are made with caution for difference in experimental condition. There are types of compounds that can stop the carbonic anhydrase II enzyme from working and they do not have sulfonamide groups either. These compounds are being studied because they might be better at targeting versions of the enzyme.

One of our compounds called compound 2h worked the best. It had an amount of 182.20 micromoles per liter. This might be because it has a part called a hydroxyl group, which is known to help compounds work against the enzyme. The way the compound is put together with a linkage might also help it work by making contact, with the enzyme in a specific way. The four compounds that were tested all showed activity against Carbonic Anhydrase II. The IC_{50} values for these compounds were between 182 and 222 µM. For comparison the reference compound acetazolamide had an IC_{50} of 388.49 µM in this assay. This is a lot higher than what has been reported in the literature for this compound.

There is a reason why this might be the case. We used bovine erythrocyte Carbonic Anhydrase II of the human version. We also used a substrate and buffer conditions. The bovine and human versions of Carbonic Anhydrase II are similar, but not identical. They share 79 percent of their sequence. The active site is very similar. There are some small differences that could affect how inhibitors bind. Therefore, caution is required when comparing IC50 value Compared the values obtained in this study with the values reported for the human Carbonic anhydrase II in pervious literature. The compounds synthesized showed inhibitory activity in our assay and further optimization of the compounds in order to increase their potency is recommended.
We also performed molecular docking studies to investigate the interaction of the synthesized compounds with active site of Carbonic anhydrase II using crystal structure (PDB ID; 1A42) retrieved from the RCSB protein bank. protein preparation and interaction visualization were carried out using Discovery studio, while ligand preparation was performed docking were subjected with Auto Dock Vina. The binding energies of compound 2g and 2e are -8.3 Kale/mol and -8.1 Kale/mol, respectively which are more favorable than that of acetazolamide (-5.8 Kale/mol). The indicate that synthesized may have good binding potential towards carbonic Anhydrase II. Docking results, though useful, are not a good predictor of inhibitory potency, especially considering possible sample size or experimental assay methodology limitations.

According, the docking finding should be interpreted as supportive evidence of binding affinity rather than definitive proof of superior biological activity over acetazolamide. The repeated involvement of residue HIS91, PHE127, VAL131, VAL139 and PRO198 across multiple ligand complexes suggested that the benzimidazole- Mannich scaffold consistency interact within the hydrophobic pocket of active site, in agreement with interaction is reported for related benzimidazole based inhibitors in the literature [16, 23]. The nitrogen containing Mannich Base functionality may further facilitate polar interaction with residue as GLN89 and HIE116. Overall compounds 2g, 2e and 2h may serve as promising lead structure for further optimization effort. We can look at the information we have. Try to understand how the structure of these compounds affects what they do. We have to remember that we only tested four out of the nine compounds to see how well they can stop CA II. When we look at the compounds, we tested compound 2h is the best at stopping CA II. This compound is made from salicylaldehyde. Has a part called diethylamine. This tells us that the hydroxyl group, which is a part of the compound is important for its activity. It probably works by forming hydrogen bonds with the water or other parts of the enzyme that have a charge.

Compound 2g, which is made from benzaldehyde and has a part called morpholine is very good at docking into the enzyme. It is not as good at stopping CA II as compound 2h. This might be because the way it actually interacts with the enzyme is different from what we predicted. We also found that compound 2f, which has morpholine in it is very good at stopping oxidation. It also stops CA II pretty well. This makes sense because we know that morpholine can help make the compound better at forming hydrogen bonds. The compound 2e, which has dimethylamine in it also did well in our tests. So what we think we are seeing is that compounds with more polar parts, especially morpholine are better at stopping radicals and compounds with phenolic aldehyde linkers might be better, at binding to CA II. All of the compounds should be tested and their effects evaluated with various classes of CA to understand what is happening.

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Conclusions

Nine new compounds that are based on 2-mercaptobenzimidazole were. Tested to see if they can stop an enzyme called CA II from working. These compounds, which are called Mannich base derivatives of 2-mercaptobenzimidazole were given the names 2a to 2i. They were checked to see if they are candidates to be taken by mouth.

All of these compounds including 2a, 2b, 2c, 2d, 2e, 2f 2g, 2h and 2i met the requirements for being absorbed by the body. Some of these compounds like 2e, 2f 2g and 2h were found to be moderately good at stopping CA II from working. This is interesting because they do not have a sulfonamide group, which's a part of many other compounds that stop CA II.

DECLARATIONS

Ethical approval: The study protocol was reviewed and approved by the Research Ethics Committee of the Riphah Institute of Pharmaceutical Sciences, Riphah International University, Islamabad, Pakistan (REC-RIPS; Ref. No. REC/RIPS/2021/23). All animal experiments were conducted in accordance with institutional and international guidelines for the care and use of laboratory animals.

Availability of data and materials: The data used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' Contributions: TS conceived and designed the study, performed the synthesis of compounds, conducted experimental work, analyzed the data, and prepared the original manuscript draft. HN supervised the research, contributed to the study design, data interpretation, and critically revised the manuscript. HH assisted in pharmacological evaluation, data analysis, and manuscript review. SMM contributed to computational studies, data interpretation, and manuscript editing. MK participated in experimental investigations, validation of results, and manuscript review. All authors read and approved the final version of the manuscript.

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REFERENCES

1. Supuran CT, Scozzafava A. Carbonic anhydrase inhibitors and their therapeutic potential. Expert Opinion on Therapeutic Patents. 2000 May 1;10(5):575-600.
2. Mishra CB, Tiwari M, Supuran CT. Progress in the development of human carbonic anhydrase inhibitors and their pharmacological applications. Med Res Rev. 2020;40(6):2485-2565.
3. Supuran CT. Advances in structure-based drug discovery of carbonic anhydrase inhibitors. Expert Opin Drug Discov. 2017;12(1):61-88.
4. Supuran CT, Scozzafava A. Carbonic anhydrases as targets for medicinal chemistry. Bioorg Med Chem. 2007;15(13):4336-4350.
5. Supuran CT. Structure and function of carbonic anhydrases. Biochemical Journal. 2016 Jul 15;473(14):2023-32.
6. Supuran CT. Carbonic anhydrase inhibitors. Bioorg Med Chem Lett. 2010;20(12):3467-3474.
7. Karataş MO, et al. Coumarin or benzoxazinone based novel carbonic anhydrase inhibitors. J Enzyme Inhib Med Chem. 2016;31(5):760-772.
8. El Ouasif L, et al. Novel 2-mercaptobenzimidazole derivatives: synthesis and evaluation of antibacterial and antioxidant activities. Mediterr J Chem. 2017;6(3):77-87.

9. Reddy SR, Kalyani P, Rao BR, Manikyamba P. Computational studies and reactivity of nucleophiles in benzylation reactions. Indian journal of chemistry. Section A, Inorganic, bio-inorganic, physical, theoretical & analytical chemistry. 2008 Feb 1;47(2):236.
10. Bala S, Sharma N, Kajal A, Kamboj S, Saini V. Mannich bases: an important pharmacophore in present scenario. Int J Med Chem. 2014.
11. Roman G. Mannich bases in medicinal chemistry and drug design. Eur J Med Chem. 2015;89:743-816.
12. Malinka W, et al. Synthesis, analgesic activity and computational study of new isothiazolopyridines of Mannich base type. Il Farmaco. 2005;60(11-12):961-968.
13. Shahnaz M, Kaur P, Parkash J, Parsad D. Synthesis, characterization of 2-substituted benzimidazole derivatives and evaluation of antimicrobial activity. J Drug Deliv Ther. 2018;8(5):460-464.
14. Gupta S, Gupta SP, Upmanyu N, Garg G. Synthesis and biological evaluation of 2-mercaptobenzimidazole derivatives as anti-inflammatory agents. J Drug Des Med Chem. 2015;1:12-6.
15. Xiao F, Xu T, Lu B, Liu R. Guidelines for antioxidant assays for food components. Food Frontiers. 2020;1(1):60-69.
16. Saadiq M, Uddin G, Latif A, Ali M, Akbar N, Ammara, Ali S, Ahmad M, Zahoor M, Khan A, Al-Harrasi A. Synthesis, bioactivity assessment, and molecular docking of non-sulfonamide benzimidazole-derived N-acylhydrazone scaffolds as carbonic anhydrase-II inhibitors. ACS omega. 2021 Dec 20;7(1):705-15.
17. Discovery Studio Visualizer v4.0.100.13345. Accelrys Software Inc. 2013.
18. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking. J Comput Chem. 2010;31(2):455-461.
19. Berman HM, et al. The protein data bank. Nucleic Acids Res. 2000;28(1):235-242.
20. Chikhi A, Bensegueni A. Docking efficiency comparison of Surflex, a commercial package and Arguslab, a licensable freeware. J Comput Sci Syst Biol. 2008;1(01):81-6.
21. Chegwidden WR, Carter ND, Edwards YH. The carbonic anhydrases: new horizons. Basel: Birkhäuser; 2000.
22. Ferreira LG, Dos Santos RN, Oliva G, Andricopulo AD. Molecular docking and structure-based drug design strategies. Molecules. 2015;20(7):13384-13421.
23. Supuran CT. Carbonic anhydrase inhibitors: an overview of the literature published in the period 2013–2018. Expert Opin Ther Pat. 2018;28(12):963-977.