An Efficient synthesis of *N*'', *N*'''-*Bis*[(*E*)-phenylmethylidene]carbonic dihydrazide derivatives and their evaluation as *α*-chymotrypsin nhibitors

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Abstract

N'',*N*'''-*Bis*[(*E*)-phenylmethylidene]carbonic dihydrazide, azotic ligands with lone electron pairs, have wide range of applications in catalysis, medicine, corrosion, and analytical chemistry. Keeping this broad range of applications in view, we have developed a new green method for the rapid synthesis of *N*'',*N*'''-*Bis*[(*E*)-phenylmethylidene]carbonic dihydrazide and its derivatives. By using a new protocol, a range of carbonic dihydrazides 1-22 has been synthesized in excellent yields. All synthesized compounds have been characterized by elemental analyses, ^{1}H-NMR, EI-MS and HR-MS. These carbonic dihydrazides have also been synthesized *via* conventional method. On comparisons of new and conventional methods, it is evident that newly developed solvent-free method is more proficient, high yielding, and simple. As protease inhibitors are known to inhibit not only proliferation of cancer cell lines but also to cure hepatitis C virus and HIV aids infections, therefore, all purified and characterized compounds have also been evaluated for their potential to act as *in vitro* *α*-chymotrypsin (an enzyme belongs to protease family) inhibitors. Among tested compounds, ten compounds have shown varying degree of *α*-chymotrypsin inhibition potential.

Keywords: *Bis*-Schiff bases; carbohydrazone; carbonic dihydrazide; *α*-chymotrypsin inhibitors; green synthesis.

1. Introduction

Hydrazones having a general formula $R_1R_2C{=}NNH_2$ constitute an imperative class of organic compounds having diverse biological activities (Haghighijoo *et al*., 2017; Mukund *et al*., 2017; Kauthale *et al*., 2017, Vanucci-Bacqué *et al*., 2016; Wang *et al*., 2015; Nasr *et al*., 2014; Pieczonka *et al*., 2013). In last decade, hydrazones owe a unique attention in various fields of

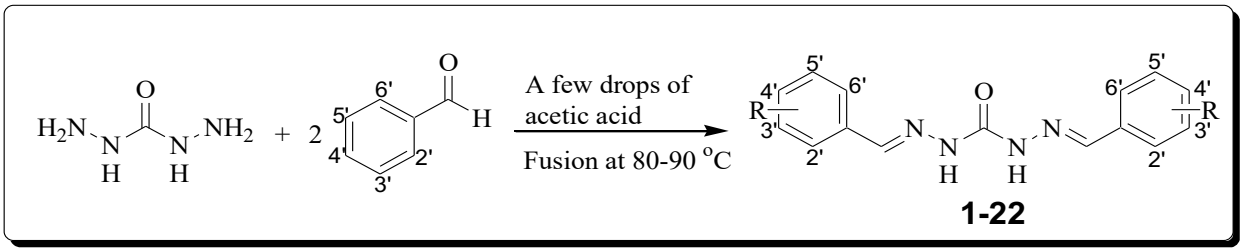
biological and chemical researchers as these compounds have ability to co-ordinate with different metals and the resulting metal complexes have been reported to possess various biological activities (Chew *et al*., 2014; Affan *et al*., 2009; Krishnamoorthy *et al*., 2011; Giziroglu *et al*., 2013). Carbohydrazide Schiff bases and their metal complexes have also been reported in the literature as therapeutic and catalytic agents (Iqbal *et al*., 2017; Singh *et al*., 2017; Taha *et al*., 2015; Chandra & Sarkar 2013; Kamyabi *et al*., 2017; Eswaran *et al*., 2010). The chelating potential of carbohydrazide Schiff bases is valuable as analytical reagent for the extraction, photometric, and fluorometric determination / quantification of transition metals (Rai *et al*., 2013; Rosales *et al*., 1985). Therefore, synthesis of carbohydrazide Schiff bases has drawn much attention of synthetic chemists. Literature search revealed that a variety of procedures have been reported for the synthesis of carbohydrazide Schiff bases. These procedures include different solvents e.g. ethanol, methanol, toluene, or tetrahydrofuran and catalysts. e.g. triethylamine, sulfuric acid, hydrochloric acid, $TiCl_2$, $Mg(ClO_4)_2$ and $ZnCl_2$ (Lefebvre *et al*., 2010; Durr *et al*., 1999; Starkov *et al*., 2017; White & Weingarten, 1967; Chakraborti *et al*., 2004; Billman & Tai, 1958). Some of these methods suffer from shortcomings such as tedious work-ups and low yields, use of special and costly catalysts. Consequently, simple, proficient, and high yielding procedures for this valuable precursor are still needed to be investigated. In current study, we have developed a new proficient procedure for the synthesis of *N*'',*N*'''-*Bis*[(*E*)-phenylmethylidene]carbonic dihydrazide (*bis*-Schiff base) derivatives without using any solvent or expensive catalyst. To compare yields and reaction time of different procedures, we carried out reaction with solvent (ethanol) as well. The comparison results have shown that solvent free method (newly developed) is high yielding, more proficient and simpler (Figures-1&2).

Alpha-chymotrypsin is a digestive enzyme and belongs to family of enzymes known as serine protease. It selectively catalyzes the cleavage of the peptide bonds with aromatic or large hydrophobic side chains as Tyr, Trp, Phe and Met, which are on the carboxyl side of this bond. It can also catalyze the hydrolysis of ester bond and a set of three amino acids i.e., histidine, aspartic acid, and serine known as catalytic triad is the catalytic driving force for *alpha*-chymotrypsin. This catalytic triad is found in the whole serine protease family and protease inhibitors are widely found in humans, plants, and other animals (Abdullah *et al*., 2021; Desalegn *et al*., 2021). Any disturbance of the balance between proteolytic enzymes and their inhibitors, or of the activation process, can result in pancreatitis. Pancreatitis carries a 40% risk of pancreatic cancer (Saima *et al*., 2005). Inhibition of proliferation of cancer cell lines has been reported by protease inhibitors (Mucsil *et al*., 2003). Chronic infection by hepatitis C virus can lead to the progressive liver injury, cirrhosis, and liver cancer. Protease inhibitors, telaprevir and boceprevir, are used as therapeutic for hepatitis C virus infection. Additionally, a combination of lopinavir/ritonavir used for the cure of HIV aids, but drug resistance and toxicity remain the issue in this regard. Therefore, it is necessary to search new protease inhibitors for drug discovery and development (Zhang *et al*., 2010; Lam & Nig, 2010). Therefore, all synthesized, purified and characterized compounds 1-22 have also been evaluated for their potential to act as *in vitro* α-chymotrypsin inhibitors.

2. Results and Discussion

2.1Chemistry

N',*N*'''-*Bis*[(*E*)-phenylmethylidene]carbonic dihydrazide and its derivatives (1-22) were synthesized by fusing carbohydrazide and a variety of aldehydes in the presence of acetic acid (Scheme-1). List of synthesized compounds 1-22 is presented in table 1.



Scheme-1: Synthesis of *N*',*N*'''-*Bis*[(*E*)-phenylmethylidene]carbonic dihydrazide and its derivatives

Typically, carbohydrazide (0.270g, 3.0mmol) and benzaldehyde (0.632ml, 6.2mmol) were grinded to make a homogeneous mixture, then 3-4 drops of acetic acid were added to this grinded mixture and heated at 80-90°C under solvent free condition. On reaction completion, the reaction mixture was allowed to cool to room temperature. Cooled reaction mixture was washed with hexane twice and crystallized from ethanol. After crystallization, the pure *N*',*N*'''-*Bis*[(*E*)-phenylmethylidene]carbonic dihydrazide and its derivatives 1-22 were obtained in excellent yields. Spectroscopic techniques (^{1}H-NMR, EI-MS, HREI-MS) were used for structure confirmation of synthesized compounds 1-22. All compounds also furnished satisfactory elemental analyses. All these compounds (1-22) were also synthesized using solvent (ethanol) and it was observed that that solvent free method is high yielding and more proficient. Comparisons of the yields and reaction times of solvent-free and solvent (ethanol) procedures are given in figures-1&2.

Table 1. List of synthesized *N*',*N*'''-*Bis*[(*E*)-phenylmethylidene]carbonic dihydrazide derivatives

Product No.	R	M.P (°C)	Product No.	R	M.P (°C)
1	H	218**°C**	**12**	2′-Cl-5′-NO_2	266**°C**
2	4′-Br	246**°C**	**13**	4′-OH	254**°C**
3	2′-Br	238**°C**	**14**	3′-OH	248**°C**
4	4′-Cl	247**°C**	**15**	2′-OH	231**°C**
5	2′-Cl	228**°C**	**16**	2′-OCH_3	210**°C**
6	2′,4′-diCl	244**°C**	**17**	3′-OCH_3	192**°C**
7	2′,6′-diCl	305**°C**	**18**	4′-OCH_3	211**°C**
8	4′-F	243**°C**	**19**	2-′OH-3′-OCH_3	207**°C**
9	2′-F	208**°C**	**20**	2′-OH-5′-OCH_3	227**°C**
10	4′-NO_2	284**°C**	**21**	2′,5′-*di*-OH	276**°C**
11	2′-NO_2	246**°C**	**22**	2′,3′,4′-*tri*-OH	259**°C**

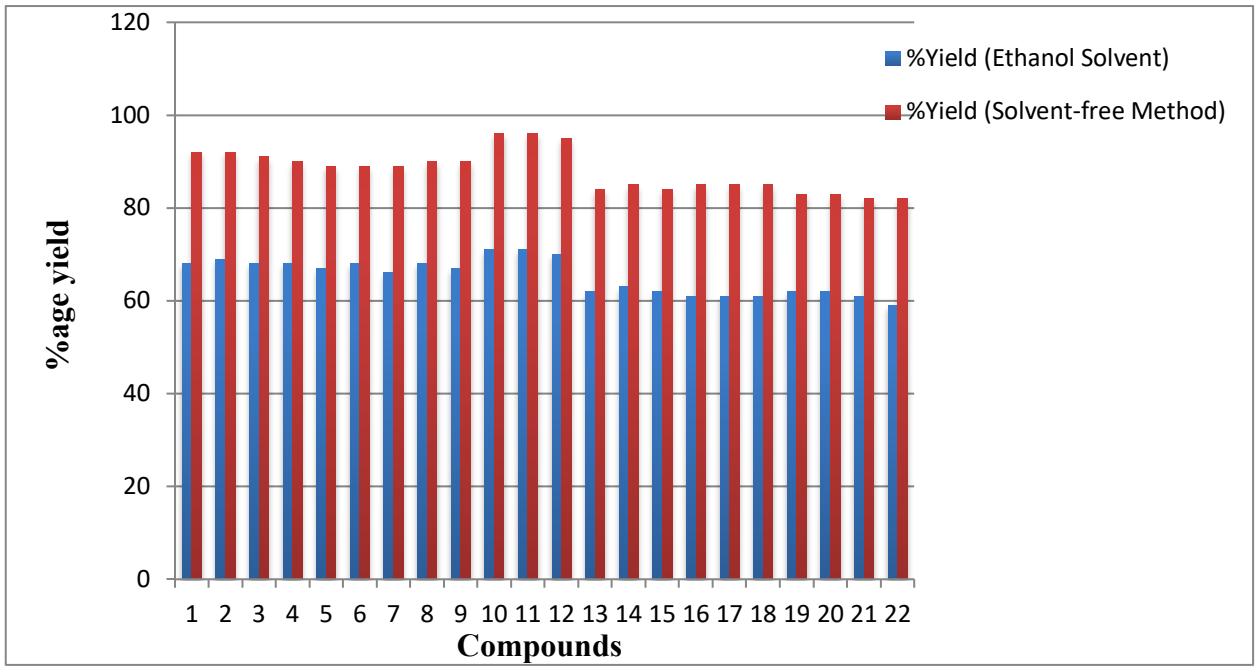


Fig. 1. Comparison of yields for synthesis performed in solvent (EtOH) and solvent-free conditions, respectively.

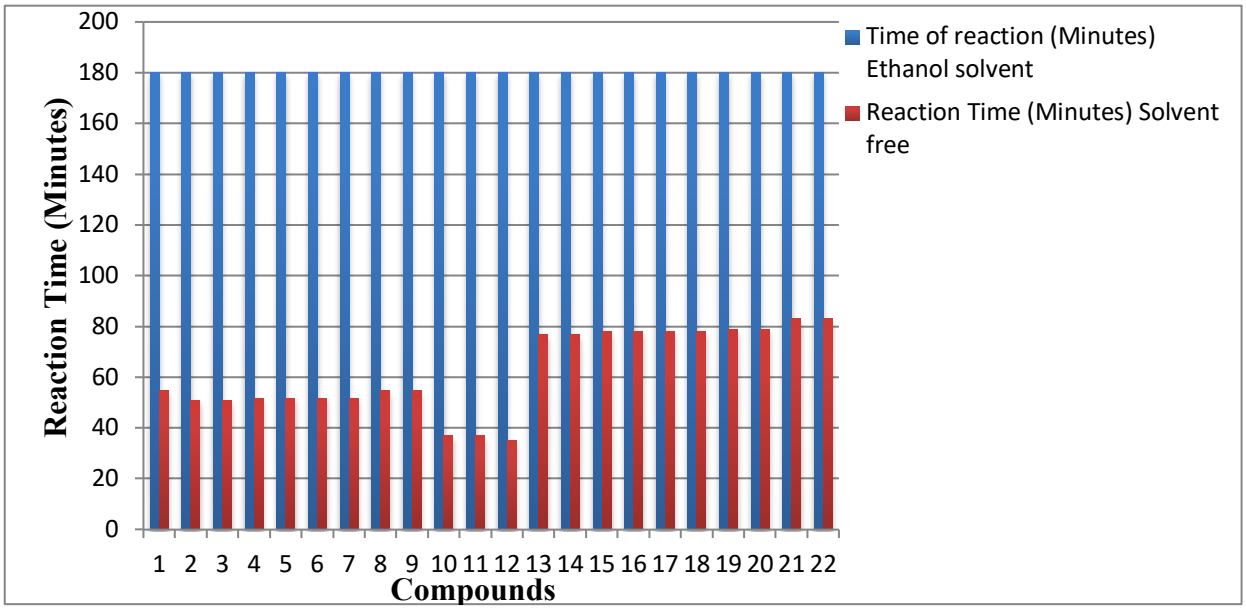


Fig. 2. Comparison of reaction time (minutes) for synthesis performed in solvent (EtOH) and solvent-free conditions, respectively.

2.2 *In vitro* α-chymotrypsin inhibition potential

Synthetic analogues 1-22 were evaluated for their *in vitro* α-chymotrypsin inhibition potential (Table-2). Among twenty-two, ten compounds showed moderate to weak inhibition potential

with IC_{50} values ranging between 100.7 ± 3.7μM - 485.7 ± 3.4μM. Compound 22 (IC_{50} = 100.7 ± 3.7μM) showed moderate inhibitory activity in comparison to standard chymostatin (IC_{50} = 5.7 ± 0.1μM). Likewise, compounds 12 (IC_{50} = 192.4 ± 2.0μM) also exhibited moderate inhibition, however compounds 2 (IC_{50} = 336.7 ± 1.2μM), 3 (IC_{50} = 324.6 ± 3.6μM), 5 (IC_{50} = 435.3 ± 7.8μM), 7 (IC_{50} = 485.7 ± 3.4μM), 8 (IC_{50} = 435.8 ± 7.0μM), 10 (IC_{50} = 452.4 ± 6.3μM), 15 (IC_{50} = 356.6 ± 0.8μM), and 20 (IC_{50} = 449.7 ± 2.3μM) showed weak inhibition potential against α-chymotrypsin. Moreover, rest of the compounds showed less than 50% inhibitory activity at 0.2mM concentration so they were not further evaluated for their IC_{50} values.

Table 2. *In vitro* α-chymotrypsin inhibition potential of compounds 1-22

Product No.	IC_{50} ± SEM[a] (μM)	Product No.	IC_{50} ± SEM[a] (μM)	Product No.	IC_{50} ± SEM[a] (μM)
1	NA[b]	**9**	NA[b]	**17**	NA[b]
2	336.7 ± 1.2	**10**	452.4 ± 6.3	**18**	NA[b]
3	324.6 ± 3.6	**11**	NA[b]	**19**	NA[b]
4	NA[b]	**12**	192.4 ± 2.0	**20**	449.7 ± 2.3
5	435.3 ± 7.8	**13**	NA[b]	**21**	NA[b]
6	NA[b]	**14**	NA[b]	**22**	100.7 ± 3.7
7	485.7 ± 3.4	**15**	356.6 ± 0.8	Chymostatin[c]	5.7 ± 0.1
8	435.8 ± 7.0	**16**	NA[b]		

SEM[a] is the standard error of the mean, NA[b] Not active. Chymostatin[c] standard inhibitor for α-chymotrypsin study

Among synthesized compounds, compound 22 having 2′,3′,4′-*tri*-OH substituents at each phenyl ring of carbohydrazone was the most active α-chymotrypsin inhibitor with IC_{50} = 100.7 ± 3.7μM. In contrast, compound 21 having 2′,5′-*di*-OH substituents at each phenyl ring of carbohydrazone has shown no activity (Figure 3).

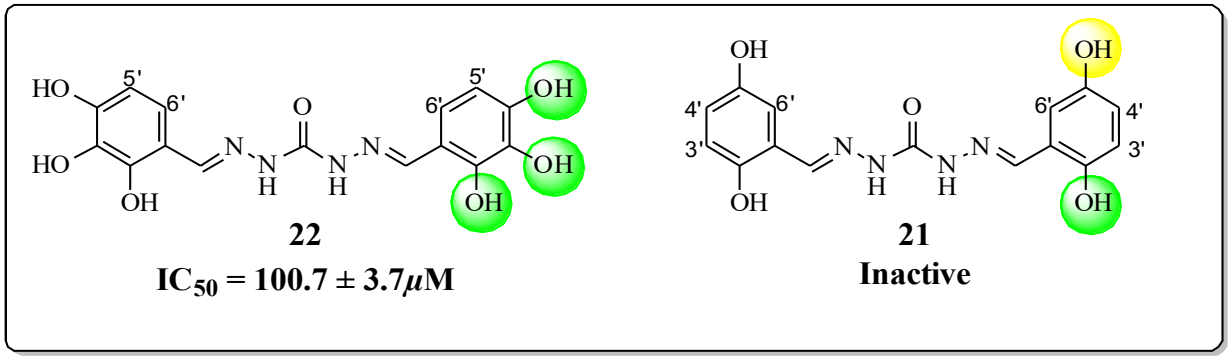


Fig. 3. A comparison of α-chymotrypsin inhibition potential of compounds having 2′,3′,4′-*tri*-hydroxy (22) and 2′,5′-*di*-hydroxy (21) substituents

Interestingly, compounds 15 and 20 having 2′-OH and 2′-OH-5-OCH_3 substituents at each phenyl ring of carbohydrazone has shown weak α-chymotrypsin inhibition with IC_{50} = 356.6 ± 0.8μM and IC_{50} = 449.7 ± 2.3μM respectively, however compound 16 having 2′-OCH_3 substituents at each phenyl ring has shown no α-chymotrypsin inhibition potential at all. It means in compound 15 and 20, hydrogen of hydroxyl substituent at C-2′ is playing an important role in α-chymotrypsin inhibition (Figure-4). If we compare IC_{50} values of compounds 2 (IC_{50} = 336.7 ± 1.2μM) and 3 (IC_{50} = 324.6 ± 3.6μM) having 4′-Br and 2′-Br substituents at each phenyl ring of carbohydrazone, it is evident that bromo substituent at C-2′ of phenyl ring is more suitable for α-chymotrypsin inhibition. Likewise, in compounds 4 (Inactive) and 5 (IC_{50} = 435.3 ± 7.8μM) having 4′-Cl and 2′-Cl substituents at each phenyl ring of carbohydrazone, chloro substituent at C-2′ of phenyl ring is more suitable for α-chymotrypsin inhibition (Figure-5). In contrast, if we compare IC_{50} values of compounds 8 (IC_{50} = 435.8 ± 7.0μM), and 9 (Inactive) having 4′-F and 2′-F substituents at each phenyl ring of carbohydrazone, it can be seen that fluoro substituent at C-4′ of phenyl ring is more suitable for α-chymotrypsin inhibition. The same trend is observed, in compounds 10 (IC_{50} = 452.4 ± 6.3μM), and 11 (Inactive) having 4′-NO_2 and 2′-NO_2 substituents at each phenyl ring of carbohydrazone, nitro substituent at C-2′ of phenyl ring is more suitable for α-chymotrypsin inhibition (Figure 6). Compound 12 having 2′-Cl-5′-NO_2 substituents at each phenyl ring of carbohydrazone is the second most active α-chymotrypsin inhibitor.

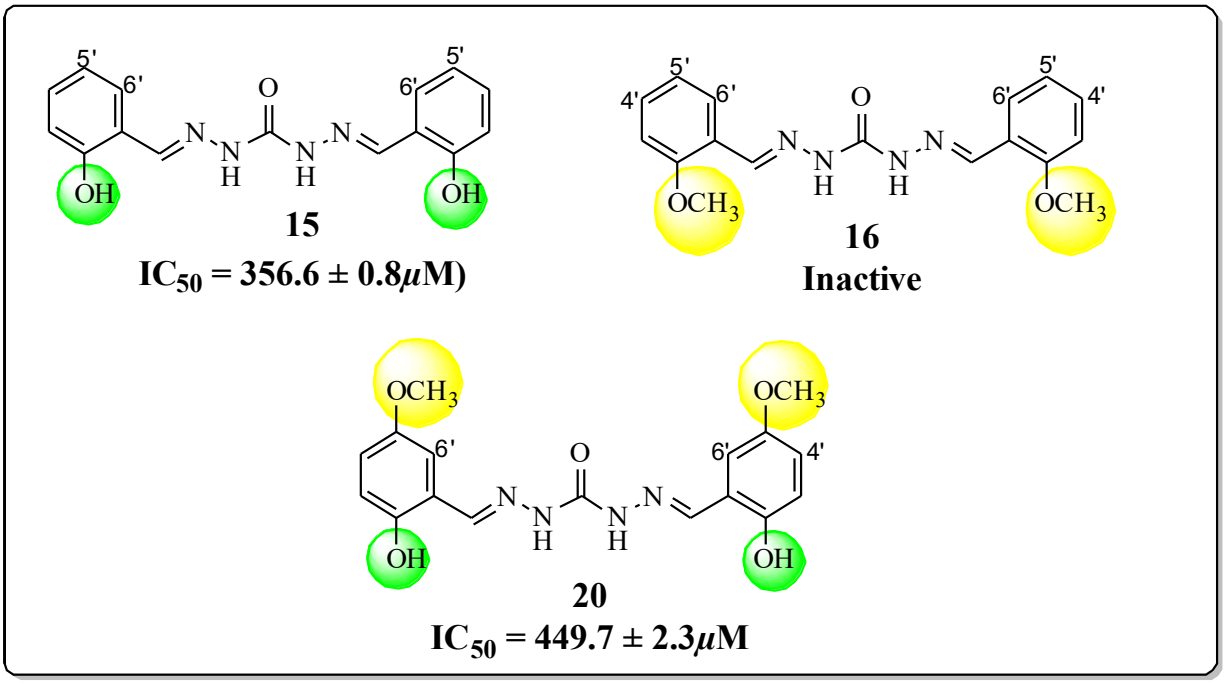


Fig. 4. A comparison of α-chymotrypsin inhibition potential of compounds having 2′-hydroxy (15), 2′-methoxy (16) and 2′-hydroxy-5-methoxy (20) substituents

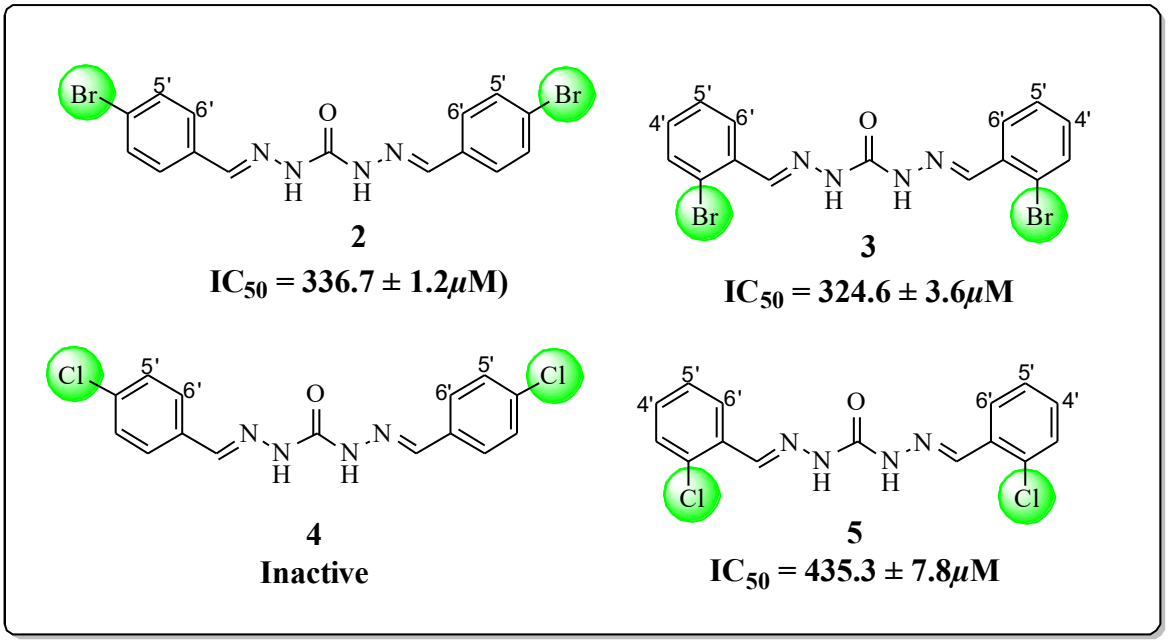


Fig. 5. A comparison of α-chymotrypsin inhibition potential of compounds having 4′-bromo (2), 2′-bromo (3), 4′-chloro (4) and 2′-chloro (5) substituents

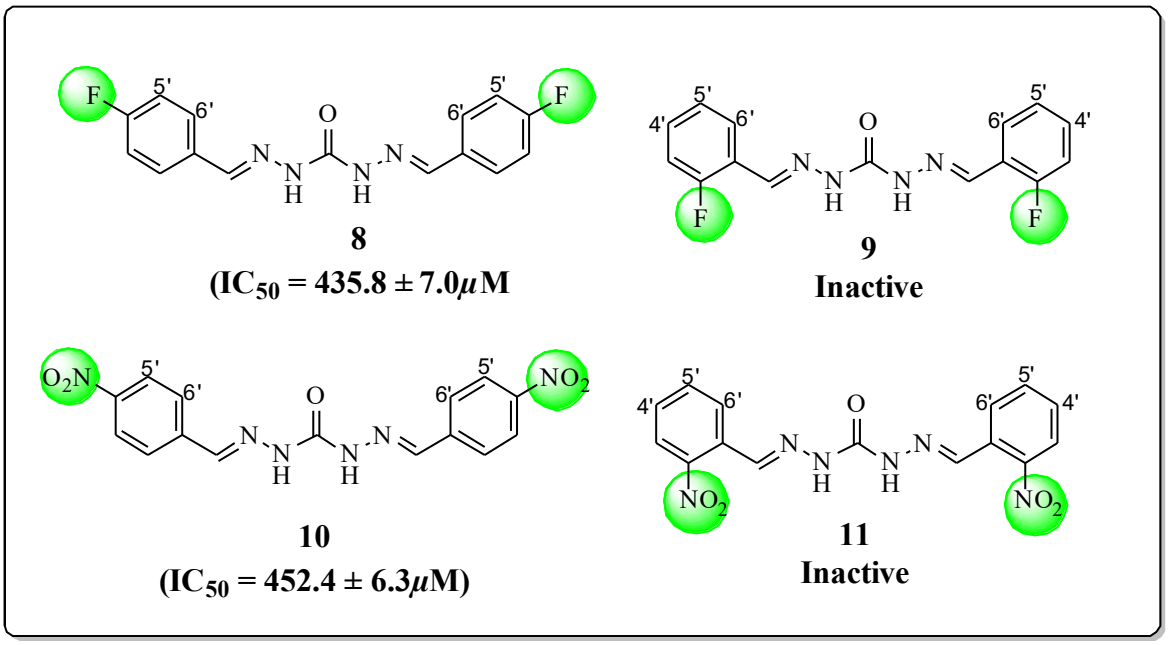


Fig. 6. A comparison of α-chymotrypsin inhibition potential of compounds having 4′-fluoro (8), 2′-fluoro (9), 4′-nitro (10) and 2′-nitro (11) substituents

From above discussion, it can be concluded, if we synthesize compounds having 2-Br′-4-F′, 2′-Br-4′-NO_2, 2-Cl′-4-F′, and 2′-Cl-4′-NO_2 substituents at each phenyl ring of carbohydrazone they may have good α-chymotrypsin inhibition potential (Figure 7).

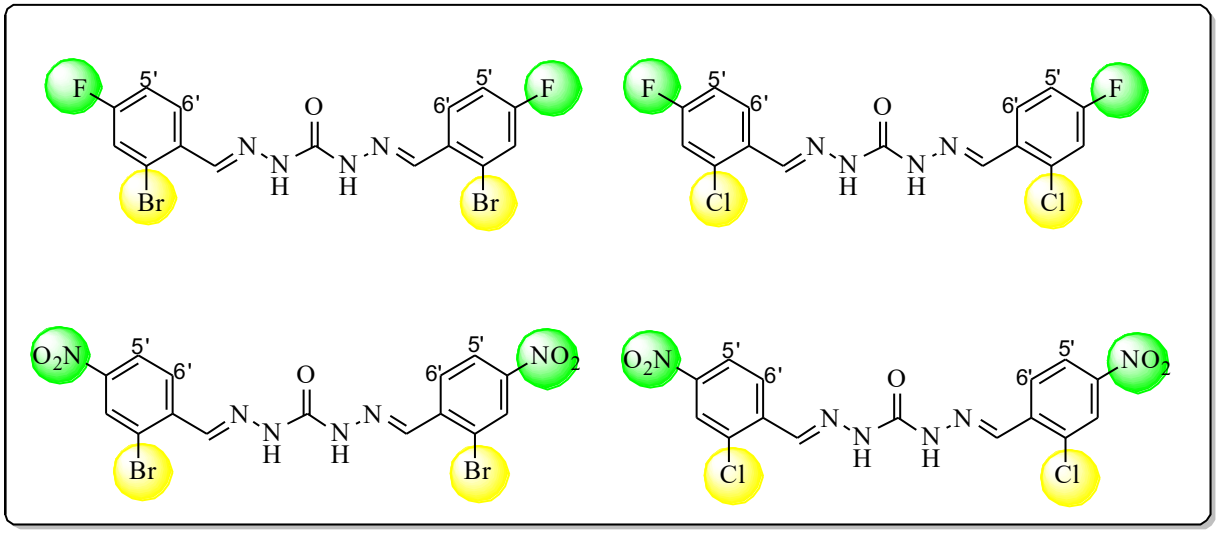


Fig. 7. New suggested compounds for tuning of α-chymotrypsin inhibition potential

3. Conclusion

Conclusively, a simple, high yielding, and proficient method for the synthesis of carbohydrazone derivatives 1-22 has been developed. The newly developed method is advantageous, as it is high yielding, require short reaction completion time. In addition, this method is inexpensive and simple as it does not require any costly or complex catalyst. All synthesized compounds 1-22 have been purified, characterized and evaluated for their ability to inhibit α-chymotrypsin, *in vitro*. Among compounds 1-22, compounds 2, 3, 5, 7, 8, 10, 12, 15, 20 and 22 have shown moderate to weak α-chymotrypsin inhibition potential. Fine tuning of the synthesized compounds may improve their α-chymotrypsin inhibition potential and lead discovery of potent α-chymotrypsin inhibitor

4. General Experimental

NMR experiments were performed on Avance Bruker AM 300, 400MHz instruments. Chemical shifts were recorded in parts per million (ppm) and coupling constants were recorded to the nearest 0.1Hz. Multiplicities are reported as singlet (s), doublet (d), triplet (t), doublet of doublets (dd), or multiplet (m). ^{13}C NMR spectra was recorded on Avance Bruker AM 75 MHz machine. Chemical shifts were recorded in parts per million (ppm). Electron impact mass spectra (EI MS) were recorded on a Finnigan MAT-311A, Germany. Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). UV light (wavelength, 365 and 254 nm) was used to visualize Chromatograms.

4.1 General procedure for the synthesis of compounds using no solvent (1-22)

N'',*N*'''-*Bis*[(*E*)-phenylmethylidene]carbonic dihydrazide and its derivatives 1-22 were synthesized by fusing carbohydrazide (0.270g. 3.0mmol) and differently substituted benzaldehydes (6.2mmol) in the presence of 3-4 drops of acetic acid without any solvent. On

reaction completion, the reaction mixture was allowed to cool at room temperature. Cooled reaction mixture was washed with hexane twice and crystallized from ethanol to get compounds 1-22 in high yields. The structures of synthetic compounds were established by ^{1}H-, ^{13}C-NMR spectroscopy, EI mass spectrometry and elemental analyses.

4.2 General procedure for the synthesis of *bis*-Schiff bases of carbohydrazide using solvent (Ethanol) (1-22)

N'',*N*'''-*Bis*[(*E*)-phenylmethylidene]carbonic dihydrazide and its derivatives 1-22 were synthesized by using method reported previously. Some of characterization data has also been reported previously (Iqbal *et al*., 2017).

Protocol for *in vitro* α-chymotrypsin Inhibition

α-Chymotrypsin inhibition potential was determined by using a literature protocol (cannell *et al*., 1988).

Availability of data and Materials

Detailed characterisation data is available as supplementary material.

Funding

This work has been funded by, "Higher Education Commission (HEC), Pakistan" under National Research Program for Universities, NRPU- Project No. 5619.

ACKNOWLEDGEMENTS

We are thankful to "Higher Education Commission (HEC), Pakistan" for providing funding under National Research Program for Universities, NRPU- Project No. 5619.

References

Abdullah, R.; Asim, M.; Nadeem, M.; Nisar, K.; Kaleem, A.; Iqtedar, M. (2021) Production, Optimization, Purification, Kinetic analysis and applications of alkaline proteases produced from *Bacillus subtilis* through solid state fermentation of agricultural byproducts. Kuwait Journal of Science (Accepted).

Affan, M. A.; Foo, S. W.; Jusoh, I.; Hanapi, S.; Tiekink, E. R. T. (2009) Synthesis, characterization and biological studies of organotin(IV) complexes with hydrazone ligand. Inorganica Chimica Acta, 362: 5031-5037.

Billman, J. H.; Tai, K. M. (1958) Reduction of schiff bases. II. Benzhydrylamines and structurally related compounds. The Journal of Organic Chemistry, 23: 535-539.

Cannell, R. J. P., Kellam, S. J.; Owsianka, A. M.; Walker, J. M. (1988) Results of a large scale screen of microalagae for the production of protease inhibitors. Planta Medica, 54, 10-14.

Chakraborti, A. K., Bhagat, S., & Rudrawar, S. (2004) Magnesium perchlorate as an efficient catalyst for the synthesis of imines and phenylhydrazones. Tetrahedron letters, 45: 7641-7644.

Chandra, S.; Sarkar, D. A. (2013) Spectral, thermal and electrochemical investigation of carbohydrazone derived ionophore as Fe(III) ion selective electrode. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 107: 271-279.

Chew, S. T.; Lo, K. M.; Lee, S. K.; Heng, M. P.; Teoh, W. Y.; Sim, K. S.; Tan, K. W. (2014) Copper complexes with phosphonium containing hydrazone ligand: Topoisomerase inhibition and cytotoxicity study. European Journal of Medicinal Chemistry, 76: 397-407.

Desalegn, T.; Bacha, K.; Tafesse, M.; Masi, C. (2021) The effectiveness of proteolytic bacteria in the leather and detergent industry isolated waste from the Modjo tannery. Kuwait Journal of Science (Accepted).

Durr, D.; Gsell, L.; Hall, R. G.; Karrer, F.; Pascual, A.; Rindlisbacher, A. (1999) Synthesis and insecticidal activity of 4-perhaloalkoxy (or thioalkyl) benzophenonehydrazone derivatives. Pesticide Science, 55: 584-587.

Eswaran, S.; Adhikari, A. V.; Pal, N. K.; Chowdhury, I. H. (2010) Design and synthesis of some new quinoline-3-carbohydrazone derivatives as potential antimycobacterial agents. Bioorganic & Medicinal Chemistry Letters, 20: 1040-1044.

Giziroglu, E.; Aygün, M.; Sarikurkcu, C.; Kazar, D.; Orhan, N.; Firinci, E.; Soyleyici, H. C.; Gokcen, C. (2013) Synthesis, characterization and antioxidant activity of new dibasic tridentate ligands: X-ray crystal structures of DMSO adducts of 1,3-dimethyl-5-acetyl-barbituric acid -hydroxybenzoyl hydrazone copper(II) complex. Inorganic Chemistry Communications, 36: 199-205.

Haghighijoo, Z.; Firuzi, O.; Hemmateenejad, B.; Emami, S.; Edraki, N.; Miri, R. (2017) Synthesis and biological evaluation of quinazolinone-based hydrazones with potential use in Alzheimer's disease. Bioorganic Chemistry, 74: 126-133.

Iqbal, S.; Saleem, M.; Azim, M. K.; Taha, M.; Salar, U.; Khan, K. M.; Perveen, S.; Choudhary, M. I. (2017) Carbohydrazones as new class of carbonic anhydrase inhibitors: Synthesis, kinetics, and ligand docking studies. Bioorganic Chemistry, 72: 89-101.

Kamyabi, M. A.; Soleymani-Bonoti, F.; Bikas, R.; Hosseini-Monfared, H. (2017) Oxygen reduction catalyzed by a Carbohydrazone based compound at liquid/liquid interfaces. Journal of Electroanalytical Chemistry, 794: 235-243.

Kauthale, S.; Tekale, S.; Damale, M.; Sangshetti, J.; Pawar, R. (2017) Synthesis, antioxidant, antifungal, molecular docking and ADMET studies of some thiazolyl Hydrazones. Bioorganic & Medicinal Chemistry Letters, 27: 3891-3896.

Krishnamoorthy, P.; Sathyadevi, P; Cowley, A. H.; Butorac, R. R.; Dharmaraj, N. (2011) Evaluation of DNA binding, DNA cleavage, protein binding and cytotoxic activities of bivalent transition metal hydrazone complexes. European Journal of Medicinal Chemistry, 46: 3376-3387.

Lam, S. K.; Nig, T. B. (2010) A dimeric high molecular weight chymotrypsin inhibitor with antitumor and HIV-1 reverse transcriptase inhibitory activities from seeds of *Acacia confuses*. Phytomedicince, 17: 621-625.

Lefebvre, V.; Cailly, T.; Fabis, F.; Rault, S. (2010) Two-step synthesis of substituted 3-aminoindazoles from 2-bromobenzonitriles. Journal of Organic Chemistry, 75: 2730-2732.

Mucsil, Z.; Gaaspaaril, Z.; Orosz, G.; Perczel, A. (2003) Structure-oriented rational design of chymotrypsin inhibitor models. Protein Engineering, 16: 673-681.

Mukund, P.; Tantak; Klingler, L.; Arun, V.; Kumar, A.; Sadana, R.; Kumar, D. (2017) Design and synthesis of *bis*(indolyl)ketohydrazide-hydrazones: Identification of potent and selective novel tubulin inhibitors. European Journal of Medicinal Chemistry, 136: 184-194.

Nasr, T.; Bondock, S.; Youns, M. (2014) Anticancer activity of new coumarin substituted hydrazide–hydrazone derivatives. European Journal of Medicinal Chemistry, 76: 539-548.

Pieczonka, A. M.; Strzelczyk, A.; Sadowska, B.; Mlostoń, G.; Stączek, P. (2013) Synthesis and evaluation of antimicrobial activity of hydrazones derived from 3-oxido-1-imidazole-4-carbohydrazides. European Journal of Medicinal Chemistry, 64: 389-395.

Rai, A.; Kumari, N.; Srivastava, A. K.; Mishra, L. (2013) Synthesis and characterization of bispentafluorophenyl carbohydrazone/thiocarbohydrazone: selective recognition of F ions. Tetrahedron Letters, 54: 7151-7155.

Rosales, D.; Gonzalez, G.; Ariza, J. L. G. (1985) Asymmetric derivatives of carbohydrazide and thiocarbohydrazide as analytical reagents. Talanta, 32: 467-474.

Saima, S.; Scott, P.; Finn, O. J.; Whitcomb, D. C. (2005) Pancreatic Cancer, Hoff, D. D. V., Ed.; Jones and Bartlett, Boston, pp. 118-138.

Singh, B.; Singh, R.; Singh, B.; Kumar, D. (2017) Computational study on molecular structure, vibrational and electronic properties of a novel Schiff base: Benzyl-2-(4-(bis(2-chloroethyl)benzylidene)hydrazinecabodithioate. Kuwait Journal of Science, 44: 98-109.

Starkov, P.; Zemskov, I.; Sillard, R.; Tsubrik, O.; Maeeorg, U. (2007) Copper-catalyzed *N*-arylation of carbamate-protected Hydrazones with organobismuthanes. Tetrahedron Letters, 48: 1155-1157.

Taha, M.; Ismail, N. H.; Javaid, K.; Imran, S.; Anouar, E. H.; Wadood, A.; Atia-tul-Wahab, Ali, M.; Khan, K. M.; Saad, S. M.; Rahim, F.; Choudhary, M. I. (2015) Evaluation of 2-indolcarbohydrazones as potent -glucosidase inhibitors, studies and DFT based stereochemical predictions. Bioorganic Chemistry, 63: 24-35.

Vanucci-Bacqué, C.; Camare, C.; Carayon, C.; Bernis, C.; Baltas, M.; Nègre-Salvayre, A.; Bedos-Belval, F. (2016) Synthesis and evaluation of antioxidant phenolic diaryl hydrazones as potent antiangiogenic agents in atherosclerosis. Bioorganic & Medicinal Chemistry, 24: 3571-3578.

Wang, Y.; Yan, H.; Ma, C.; Lu, D. (2015) Synthesis and anticancer activities of novel 8-azapurine carbocyclic nucleoside Hydrazones. Bioorganic & Medicinal Chemistry Letters, 25: 4461-4463.

White, W. A.; Weingarten, H. (1967) A versatile new enamine synthesis. The Journal of Organic Chemistry, 32: 213-214.

Zhang, C.; Zhou, D.; Zheng, S.; Liu, L., Tao, S.; Yang, L.; Hub, S.; Feng, Q. A. (2010) Chymotrypsin-like serine protease cdna involved in food protein digestion in the common cutworm, *spodoptera litura*: cloning, characterization, and induced expression patterns, and localization. Journal of Insect Physiology, 56: 788-799.

Submitted : 06/11/2021
Revised : 15/02/2022
Accepted : 20/02/2022
DOI : 10.48129/kjs.19975