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PII: S0960-894X(15)00643-5
DOI: http://dx.doi.org/10.1016/j.bmcl.2015.06.050
Reference: BMCL 22840

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 28 December 2014
Revised Date: 29 May 2015
Accepted Date: 12 June 2015

Please cite this article as: Doddaramappa, S.D., Lokanatha Rai, K.M., Srikantamurthy, N., Chandra, Chethan, J., Novel 5-functionalized-pyrazoles: Synthesis, characterization and pharmacological screening, *Bioorganic & Medicinal Chemistry Letters* (2015), doi: http://dx.doi.org/10.1016/j.bmcl.2015.06.050

Graphical Abstract

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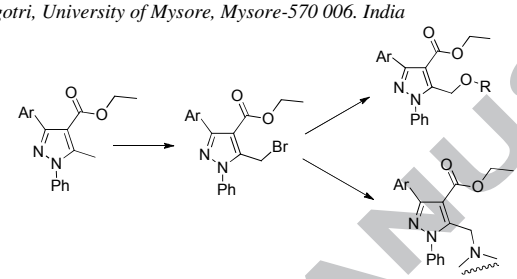

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Bioorganic & Medicinal Chemistry Letters
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ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
Pyrazole-4-carboxylate,
NBS, *O*-substitution,
N-substitution,
phase transfer catalysis,
TBAB.

ABSTRACT

In the present study a series of *O*-substituted pyrazoles **7**(**a–f**) and *N*-substituted pyrazoles **9**(**a–f**) were synthesized *via* phase-transfer catalyzed reaction of ethyl 5-(bromomethyl)-1,3-diphenyl-1*H*-pyrazole-4-carboxylate **5** with various oxygen and nitrogen containing compounds in presence of tetrabutylammonium bromide (TBAB) in THF. The compound **5** was obtained by the efficient bromination with *N*-bromosuccinimide (NBS) in presence of a catalytic amount of azoiso-bis-butyro nitrile (AIBN) in refluxing CCl_4. The synthesized compounds were evaluated for their *in vitro* antimicrobial and antidiabetic activity and were compared with standard drugs. Among the synthesized compounds, compound **9b** emerged as an excellent antimicrobial and antidiabetic agent. Newly synthesized compounds were characterized by analytical and spectral (IR, ^{1}H NMR, ^{13}C NMR and LC–MS) methods.

Due to the increased rate of microbial infections[1] and resistance to antimicrobial agents,[2] identification of novel structure leads that may be of use in designing new, potent and broad spectrum antimicrobial agents remains a major challenge for medicinal chemistry researchers. Thus, intense efforts in antimicrobial drug discovery is still needed to develop more promising, economical and effective drugs for use in the clinical arena.[3]

DM (Diabetes mellitus) is a metabolic disorder characterized by chronic hyperglycemia or increased blood glucose levels with disturbances in carbohydrate, fat and protein metabolism resulting from absolute or relative lack of insulin secretion.[4] Diabetes, being one of the most common global diseases, affects approximately 200 million individuals worldwide and approximately 300 million people worldwide are at risk of diabetes.[5] The management of the blood glucose level is a critical strategy in the control of diabetes complications. It is widely accepted that the most challenging goal in the management of patients with diabetes mellitus is to maintain blood glucose levels as close to normal as possible.

The inhibition of enzymes involved in the digestion of carbohydrates can significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet by delaying the process of carbohydrate hydrolysis and absorption. The control of postprandial hyperglycemia is an important strategy in the management of diabetes mellitus, especially type II diabetes and reducing chronic complications associated with the disease. Therefore, such enzyme inhibitors can be useful in the treatment of type II diabetes.[6]

Pyrazole is a five membered ring system with two nitrogen atom represents an important class of compounds not only for their theoretical interest but also for anti-inflammatory, analgesic, antitumor, anti hypertensive, antipyretic, sedatives, antibacterial and antidiabetic activities.[7,8] In fact, some of the pyrazole derivatives like Celecoxib, Viagra, Fipronil etc., are now widely used in the market as therapeutic agents.[9-13]

In the light of these facts and in continuation of our interest in the synthesis of heterocycles containing a multi-structure for biological activity[14] we thought of synthesizing a new class of 5-functionalized-pyrazole, to see the additive effect of these rings towards the *in vitro* antimicrobial and antidiabetic activity, which is the current passion being accomplished in most of the drug discoveries.[15,16]

The synthesis of title compounds ethyl 5-(*O*-alkyl-substituted)-1,3-diphenyl-1*H*-pyrazole-4-carboxylate derivatives **7**(**a–f**) and 5-(*N*-alkyl-substituted)-1,3-diphenyl-1*H*-pyrazole-4-carboxylate derivatives **9**(**a–f**) were prepared as shown in **Scheme 1** and **Scheme 2** respectively. The crucial compound

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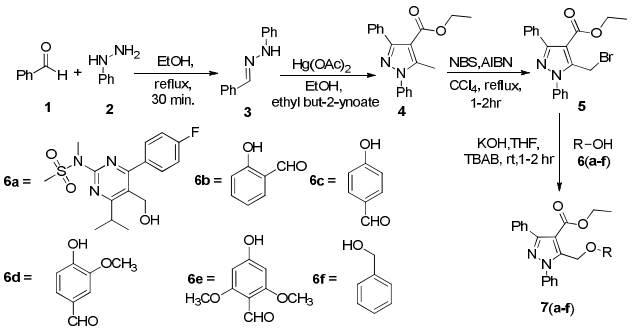
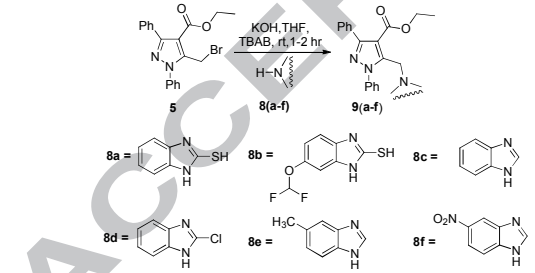
ethyl-5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carboxylate **4** was synthesized regioselectively,[17] which involves mild one pot reaction of benzaldehyde and phenyl hydrazine with ethyl but-2-ynoate in presence of $Hg(OAc)_2$ and EtOH as the solvent.

In the next step bromination of methyl group attached to the 5th position of the pyrazole **4** was achieved by using *N*-bromosuccinimide (NBS) as a brominating agent[18] in the presence of a catalytic amount of a free-radical initiator such as AIBN in CCl_4 under reflux. This procedure works well giving good yield of corresponding brominated pyrazole **5**.



Scheme 1: Synthesis of *O*-alkylated pyrazoles **7**(**a–f**)

In order to evaluate biological activity of different oxygen and nitrogen containing pyrazole derivatives, in the last step we have carried out *O*-alkylation and *N*-alkylation of brominated pyrazole **5** with various hydroxyl bearing compounds **6**(**a–f**) and secondary amines **8**(**a–f**) in presence of tetrabutyl ammonium bromide (TBAB) and KOH in THF as the solvent to yield ethyl 5-(*O*/*N*–substituted)-1,3-diphenyl-1*H*-pyrazole-4-carboxylate derivatives **7(a–f)** and **9**(**a–f**). The *N*-(6-(4-fluorophenyl)-5-(hydroxymethyl)-4-isopropyl-4,5-dihydropyrimidin-2-yl)-*N*-methylmethanesulfonamide, a component of *Rosuvastatin* which is used as dislypidemia **(6a)** was a gift from my Professor, compound **8a** and **8b** were synthesized from the existing literature[19,20] The remaining hydroxyl bearing compounds **6**(**b–f**) and amines **8(c-f)** were obtained from commercial suppliers and used without further purification. Alkylation was carried out by using Phase transfer catalysis (PTC).[19] PTC is a relatively new method for the promotion[20,21] of two-phase reactions. Afresh synthesized compounds were characterized by analytical and spectral methods.



Scheme 2: Synthesis of *N*-alkylated pyrazoles **9**(**a–f**)

The structural assignments to newly synthesized compounds **7(a–f)** and **9**(**a–f**) were based on their elemental analysis and spectral (IR, ^{1}H NMR, ^{13}C NMR and Mass) data. The ^{1}H NMR spectra of the compound **5a** showed disappearance of peaks at δ 1.15 (3H, s) $–CH_3$ protons and appearance of singlet due to $–CH_2Br$ of ethyl 5-(bromomethyl)-1,3-diphenyl-1*H*-pyrazole-4-carboxylate at δ 4.83 confirms the formation of product. Similarly the appearance of a new singlet peak at δ 4.16 due to $–CH_2–O–$ and δ 4.15 due to $–CH_2–N<$ confirms the formation of novel ethyl 5-Substituted-1,3-diphenyl-1*H*-pyrazole-4-carboxylate **7a** and **9a**. In ^{13}C NMR spectra, absence of peak at δ 11.5 ($–CH_3$) and 20.2 ($–CH_2Br$) of **4** and **5** and appearance of peak at 65.9 and 47.9 due to $–CH_2–O–$ and $–CH_2–N<$ substantiated the formation of compounds **7a** and **9a**.

The newly synthesized compounds **7**(**a–f**) and **9**(**a–f**) were screened *in vitro* for their antibacterial activity against four bacterial species, *viz*: *Bacillus cereus* (NCIM, 2016; MTCC 8372), *Staphylococcus aureus* (NCIM, 2079; MTCC 96), (gram-positive bacteria), *Escheria coli* (NCIM, 2065; MTCC 724), *Klebsiella pneumonia* (NCIM, 2957; MTCC 3384), (gram-negative bacteria) and antifungal activity against two fungal species, *viz*: *Aspergillus flavus* (MTCC 873), *Aspergillus niger* (MTCC 281), by disc diffusion[22] and microdilution method.[23] The antibiotic *Tetracycline* and *Nystatin* were used as positive reference to determine the sensitivity of each microbial species tested. The smallest amount of synthesized compounds or standard (*Tetracycline*) antibiotic was required to inhibit the visible growth of a test microorganism (MIC) and the lowest concentration of an antibiotic required to kill a particular bacterium/fungi (MBC/MFC). The results are compiled in **Table 1–2**. In all the determinations tests were performed in six replicate and the results were taken as a mean of at least three determinations.

All compounds exhibit good to potent *in vitro* antimicrobial activity against Gram-positive and Gram-negative stains. The results revealed that, compounds **7a**, **7e**, **9a**, **9b**, **9d** and **9f** exhibit excellent antibacterial activity. The compound **7a** was active against gram positive while the compound **7e** showed better activity against gram negative strain. Among the compounds **9**(**a–f**), compound **9b** emerged as a promising broad spectrum anti-bacterial agent, while the fungal strains were inhibited by the compounds **7a**, **9a**, **9d** and **9e**. The compound **9a** which has thiol group shows better activity than **9d** which contain chloro group. The compound **9e** containing electron donating $–CH_3$ group was less active against bacterial strains but possess good antifungal activity while the compound **9f** containing electron withdrawing $–NO_2$ group was less active against both the fungal strains. Compare to the *O*-substituted-pyrazole derivatives, the *N*-substituted pyrazolyl-benzimidazole derivatives are very potent and this was attributed to the presence of benzimidazole ring.

α-Amylase and α-glucosidase are important enzymes which catalyses the hydrolysis of carbohydrates into simpler monosaccharides that are absorbed in the small intestine. The inhibition of these enzymes slow down the process of absorption of glucose decomposed from starch by these enzymes there by control the diabetes.[24-26] Therefore, efficient inhibitors of α-amylase and α-glucosidase have long been sought.

In this study we have synthesized the new molecules **7**(**a–f**) and **9**(**a–f**) and were screened *in vitro* for their antidiabetic activity by measuring the α-amylase and α-glucosidase inhibitory potential. The IC_{50} values of tested compounds **7**(**a–f**) and **9**(**a–f**) on α-amylase and α-glucosidase are showed in **Table 3**.

Among the compounds tested for antidiabetic activity, the compounds **7a**, **9b** and **9d** emerged as a potent inhibitor of both the enzymes. This may be due to the presence of dihydro-pyrimidine ring in **7a** which is a part of bioactive oral drug *Rosuvastatin* for lowering blood cholesterol levels. While the compound **9b** which contains difluoromethoxy group and **9d** which contains -Cl group exhibits promising antidiabetic activity. This result was also supported by the molecular docking studies as discussed below. For simplicity, we report the docking poses for the most active compounds **7a**, **9b** and **9d** only (Fig. 1).

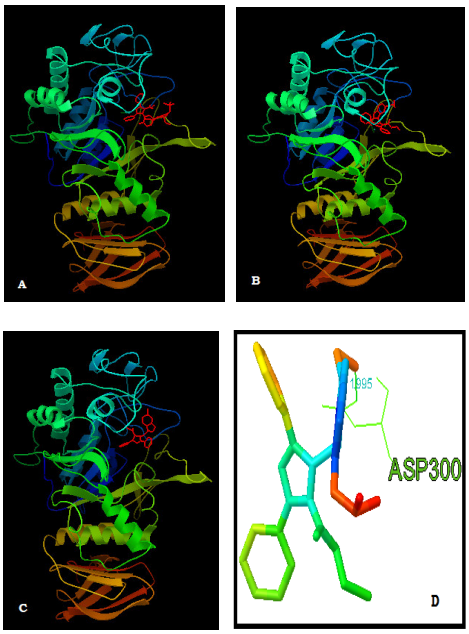


Figure 1 Docking of (A) **7a**, (B) **9b**, (C) **9d** against *α*-amylase and (D) **9b** showing hydrogen bond.

Rational drug design helps to expedite the drug designing process, which involves variety of methods to identify novel compounds. One such method is the docking of the drug molecule with the receptor. In order to gain more insight into the interaction between these new series of compounds **7(a–f)** and **9(a–f)** with *α*-amylase and *α*-glucosidase molecular docking studies were performed. As *in vitro* study of compounds **7(a–f)** and **9(a–f)** showed high inhibition activity against *α*-amylase when compared to *α*-glucosidase, *α*-amylase was selected for molecular docking study.

Automated docking was used to assess the binding modes and conformation of the ligand molecules. Molecular docking study is a well-established technique to determine the interaction of two molecules and find the best orientation of ligand would form a complex with overall minimum energy. All the compounds **7(a–f)** and **9(a–f)** were found to have minimum binding energy ranging from -6.32 to -8.68 kJ/mol with *α*-amylase (PDB Code: 1PPI) (**Table 4**). Among the molecules tested for docking study, the compound ethyl 5-((6-(difluoromethoxy)-2-mercapto-1*H*-benzo[d]imidazol-1-yl)methyl)-1,3-diphenyl-1*H*-pyrazole-4-carboxylate **9b** showed minimum binding energy -8.60 kJ/mol with ligand efficiency of -0.23 which is due to dipole-dipole and hydrogen bond interaction with the targeted protein. The 1PPI comprises of twenty one active site residues, which are promiscuous to the ligands. Out of which GLN 63, HIS 305, LYS 200, GLY 306 and ASP 300 residues are directly interacting with the ligands (**7b**, **7c**, **7d**, **7e**, **7f**, **9b** and **9f**), where as the ligands **7a**, **9a**, **9c**, **9d** and **9e** has no hydrogen bond interaction with the 1PPI. Most of the residues that are in close proximity to the inhibitor are hydrophobic in nature. The docking study results showed that the compounds **7(a–f)** and **9(a–f)** have good inhibition constants. The details of docked score results of the compounds with *α*-amylase are given in the **Table 4** (see supplementary material).

In conclusion, we have synthesized a new series of "5-(*O*/*N*-substituted)methylpyrazoles **7(a–f)** and **9(a–f)** and these compounds were investigated for their *in vitro* antimicrobial and antidiabetic activity. Subsequently, these novel classes of compounds have emerged as potent antibacterial and antifungal agents. Among the synthesized compounds, compound **7a**, **9b** and **9d** showed excellent antimicrobial and antidiabetic activity in comparison with standard drugs in *in vitro* studies. The SAR study of the title compounds inferred that, the bio-activity of these compounds are strongly dependent on the nature of the substituents at the ether linked aryl ring attached to the pyrazole unit, along with the substituent linked to the benzimidazole unit. *In vivo* and cytotoxicity investigations of the best active compounds **7a**, **9b** and **9d** are necessary to fully appraise the potential of these compounds.

Acknowledgments

One of the authors (D.S.D) is grateful to Rajiv Gandhi National Fellowship (DV5/130(6)/RGNF/2011-2012 dated 08-08-2011) UGC, New Delhi, for providing the necessary fund to carry out the research at University of Mysore.

Supplementary Material

Supplementary data (Experimental details, NMR, MS and elementary analysis) associated with this article can be found, in the online version, at

References and notes

1. Peara, S.; Patterson, T. F. *Clin. Infect. Dis.* **2002**, *35*, 1073.
2. Giulia, M.; Luisa, M.; Paola, F.; Silvia, S.; Angelo, R.; Luisa, M.; Francesco, B.; Roberta, L.; Chiara, M.; Valeria, M.; Paolo, L. C.; Elena, T. *Bioorg. Med. Chem.* **2004**, *12*, 5465.
3. Vincent, T. A.; *J. Antimicrob. Chemother.* **1999**, *44*, 151.
4. World Health Organization Consultation: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Report of a WHO Consultation Geneva; 1999.
5. Mc Cune, L. M.; Johns, T. *J Ethnopharmacol.* **2002**, *82*, 197.
6. Rhabasa L. R.; Chiasson, J. L. *α-glucosidase inhibitors*; In Defronzo, R. A.; Ferrannini, E.; Keen, H.; Zimmet, P. (Eds.). International textbook of diabetes mellitus, 3rd ed.; John Wiley: UK, Vol. 1, 2004.
7. Wustrow, D. J.; Capiris, T.; Rubin, R.; Knobelsdorf, J. A.; Akunne, H.; Davis, M. D.; MacKenzie, R.; Pugsley, T. A.; Zoski, K. T.; Heffner, T. G.; Wise, L. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2067.
8. Penning, T. D.; Talley, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Docter, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogier, D. J.; Yu, S. S.; Anderson, G. D.; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Zhang, Y. Y.; Isakson, P. C. *J. Med. Chem.* **1997**, *40*, 1347.
9. Terrett, N. K.; Bell, A. S.; Brown, D.; Ellis, P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1819.
10. Gang, S.; Pengfei, L.; and Yu Rao. *Org. Lett.* **2011**, *13*, 1746.
11. Strecker, A.; Justus Liebigs *Ann. Chem.* **1850**, *75*, 27.
12. Domling, A.; Ugi, I. *Angew. Chem., Int. Ed.* **2000**, *39*, 3168.
13. Kappe, C. O. *Acc. Chem. Res.* **2000**, 33, 879.
14. Ningaiah, S.; Bhadraiah, U. K.; D. Shridevi Doddaramappa Keshavamurthy, S.; Javarasetty, C. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 245.
15. Zhou, C. H.; Wang, Y.; *Current Medicinal Chemistry.* **2012**, *19*, 239.
16. Wang, Y.; Zhou, C. H.; *Science Sinica Chimica.* **2011**, *41* 1429.
17. Srikantamurthy, N.; Shridevi Doddaramappa, D.; Chandra, Mahendra, M.; Shubakara, K.; Umesha, K. B. *Synthetic Commun,* **2014**, *44*, 2222.
18. Almansa, C.; Gomez, L. A.; Cavalcanti, F. L.; Arriba, A. F.; Rafanell, J. D.; Form, J. G. *J. Med. Chem.* **1997**, *40*, 547.
19. Van Allan, Deacon, *Org.Syn coll.vol.IV*, **1963**, 569.
20. Sharada, L. N.; Satyanarayanareddy, G.S.; Sammaiah, B.; Sumalatha, D. *Asian Journal of Chemistry,* **2013**, 25 (14), 7959.
21. The optimized reaction condition of PTC-alkylation: ethyl 5-(bromomethyl)-1,3-diphenyl-1*H*-pyrazole-4-carboxylate **5** were

treated with different *O/N* containing compounds in a 1:1 molar ratio in THF as a liquid phase and potassium hydroxide as a basic solid phase in the presence of TBAB as a catalyst. The reaction needs vigorous and efficient stirring and the reaction progress was monitored by thin-layer chromatography (TLC).

22. O'Donnell, M. J.; in: I. Ojima (Ed.), *Catalytic Asymmetric Synthesis*, VCH Publishers, New York, Chapter 8, p. 389, 1993.
23. Shioiri, T.; in: Y. Sasson, R. Neumann (Eds.), *Handbook of Phase-Transfer Catalysis*, Blackie Academic & Professional, London, Chapter 14, 1997.
24. Andrews, J. M. *J. Antimicrob. Chemother.* **2008**, *62*, 256.
25. Zgoda, J. R.; Porter, J. R. *Pharmaceutical Biology*. **2001**, *39*, 221.
26. Hara, Y.; Honda, M. *Agricultural and Biological Chemistry*, **1990**, *54*, 1939.
27. Layer, P.; Rizza, R. A.; Zinsmeister, A. R.; Carlson, G. L.; Di Magno, E. P. *Mayo Clin Proc.* **1986**, *61*, 442.
28. Tundis, R.; Loizzo, M. R.; Menichini, F. *Mini Rev. Med. Chem.* **2010**, *10*, 315.

Tables

Novel 5-functionalized-pyrazoles: Synthesis, characterization and pharmacological screening

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Table 1 Inhibitory zone[a] (diameter) mm of synthesized compounds **7(a-f)** and **9(a-f)** against tested microbial strains

Compounds	Antibacterial activity								Antifungal activity			
	Gram positive				Gram negative							
	B. cereus		*S. aureus*		*E. coli*		*K. pneumonia*		*A. flavus*		*A. niger*	
	50 µg/ml ± SD	**100** µg/ml ± SD	**50** µg/ml ± SD	**100** µg/ml ± SD	**50** µg/ml ± SD	**100** µg/ml ± SD	**50** µg/ml ± SD	**100** µg/ml ± SD	**50** µg/ml ± SD	**100** µg/ml ± SD	**50** µg/ml ± SD	**100** µg/ml ± SD
7a	08 ± 0.18	20 ± 0.11	06 ± 0.50	19 ± 0.10	03 ± 0.08	10 ± 0.08	08 ± 0.11	10 ± 0.25	12 ± 0.11	06 ± 0.14	10 ± 0.50	08 ± 0.09
7b	04 ± 0.12	16 ± 0.13	02 ± 0.18	12 ± 0.02	03 ± 0.11	08 ± 0.11	06 ± 0.21	09 ± 0.24	10 ± 0.15	05 ± 0.11	10 ± 0.10	10 ± 0.12
7c	03 ± 0.10	03 ± 0.06	02 ± 0.24	08 ± 0.53	10 ± 0.05	16 ± 0.07	08 ± 0.11	12 ± 0.11	20 ± 0.14	05 ± 0.06	12 ± 0.42	10 ± 0.22
7d	04 ± 0.13	08 ± 0.27	02 ± 0.13	08 ± 0.16	03 ± 0.15	08 ± 0.11	04 ± 0.22	06 ± 0.08	08 ± 0.12	09 ± 0.05	12 ± 0.55	10 ± 0.35
7e	06 ± 0.11	10 ± 0.11	08 ± 0.16	10 ± 0.17	15 ± 0.07	30 ± 0.25	10 ± 0.10	19 ± 0.11	10 ± 0.22	12 ± 0.08	14 ± 0.48	11 ± 0.28
7f	02 ± 0.09	08 ± 0.25	02 ± 0.17	06 ± 0.24	04 ± 0.25	06 ± 0.22	03 ± 0.12	06 ± 0.19	08 ± 0.11	10 ± 0.18	12 ± 0.69	08 ± 0.44
9a	08 ± 0.12	11 ± 0.21	08 ± 0.13	12 ± 0.16	08 ± 0.23	16 ± 0.09	08 ± 0.52	14 ± 0.18	14 ± 0.28	18 ± 0.15	10 ± 0.19	16 ± 0.46
9b	**11 ± 0.07**	**26 ± 0.05**	**12 ± 0.02**	**21 ± 0.17**	**20 ± 0.19**	**33 ± 0.08**	**16 ± 0.19**	**28 ± 0.21**	**18 ± 0.16**	**20 ± 0.11**	**12 ± 0.11**	**22 ± 0.08**
9c	06 ± 0.09	12 ± 0.16	06 ± 0.04	12 ± 0.19	08 ± 0.15	10 ± 0.18	06 ± 0.16	10 ± 0.12	12 ± 0.12	15 ± 0.12	25 ± 0.19	20 ± 0.15
9d	06 ± 0.11	14 ± 0.13	09 ± 0.42	11 ± 0.10	09 ± 0.11	19 ± 0.14	06 ± 0.10	12 ± 0.21	08 ± 0.20	09 ± 0.11	10 ± 0.20	10 ± 0.22
9e	08 ± 0.12	11 ± 0.19	06 ± 0.36	12 ± 0.20	08 ± 0.30	12 ± 0.13	09 ± 0.11	11 ± 0.26	16 ± 0.19	21 ± 0.15	12 ± 0.18	24 ± 0.13
9f	05 ± 0.18	10 ± 0.17	06 ± 0.22	12 ± 0.11	08 ± 0.18	18 ± 0.08	06 ± 0.12	11 ± 0.22	06 ± 0.14	10 ± 0.13	05 ± 0.16	10 ± 0.41
Tetracycline	10 ± 0.11	22 ± 0.19	10 ± 0.11	20 ± 0.10	18 ± 0.11	30 ± 0.15	12 ± 0.18	20 ± 0.19	-----	-----	-----	-----
Nystatin	-----		-----		-----		-----		16 ± 0.18	20 ± 0.08	10 ± 0.36	20 ± 0.36

[a] Zone of inhibition (Mean six replicate ± standard deviation).

Table 2 The minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) in µg/mL of synthesized compounds **7(a-f)** and **9(a-f)** against tested strains

Compounds	Antibacterial activity[a]								Antifungal activity[a]			
	Gram positive				Gram negative							
	B. cereus		*S. aureus*		*E. coli*		*K. pneumonia*		*A. flavus*		*A. niger*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC
7a	15	150	40	190	35	280	30	240	55	300	50	280
7b	45	250	55	220	65	250	85	260	50	270	50	225
7c	40	210	40	210	55	235	55	230	40	275	45	260
7d	45	230	45	240	40	210	45	200	40	250	40	205
7e	40	200	40	200	25	160	20	150	50	215	45	190
7f	50	255	40	210	45	210	45	230	55	270	45	265
9a	20	160	20	150	25	175	25	160	50	290	40	290
9b	10	130	20	120	15	135	15	130	30	155	25	145
9c	60	285	55	290	55	270	40	220	45	275	50	280
9d	35	220	30	160	35	190	30	190	30	230	25	215
9e	50	220	45	205	40	205	45	240	40	270	45	165
9f	35	200	25	190	25	180	25	185	55	290	65	280
Tetracycline	4	120	10	120	12	120	8	120	---	---	---	---
Nystatin	---	---	---	---	---	---	---	---	08	100	10	100

[a] (Mean six replicate ± standard deviation).

Table 3 Antidiabetic Activity[a] of synthesized compounds **7(a-f)** and **9(a-f)**

product	IC_{50} values of α-amylase inhibition activity	IC_{50} values of α-glucosidase inhibition activity
7a	15 µg/ml	25 µg/ml
7b	40 µg/ml	40 µg/ml
7c	60 µg/ml	65 µg/ml
7d	60 µg/ml	70 µg/ml
7e	40 µg/ml	35 µg/ml
7f	45 µg/ml	45 µg/ml
9a	40 µg/ml	45 µg/ml
9b	10 µg/ml	15 µg/ml
9c	35 µg/ml	30 µg/ml
9d	10 µg/ml	20 µg/ml
9e	45 µg/ml	45 µg/ml
9f	30 µg/ml	30 µg/ml
Acarbose (+ ve control)	15 µg/ml	15 µg/ml

[a] Each value represents a mean of three replicates

Table 4 The dock score results of synthesized compounds **7(a-f)** and **9(a-f)** with α-amylase (PDB Code: 1PPI)

Compounds	Binding Energy (kJ mol^{-1})	Ligand Efficiency	Inhibition Constant	vdW+H-bond+desolv energy	No. of H- bonds	Bonding residues	Bond Length (Å)
7a	-6.32	-0.13	23.34	-9.97	-	-	
7b	-7.56	-0.24	2.86	-9.83	1	1PPI:A:GLN63:HE22	1.743
7c	-7.94	-0.25	1.51	-9.79	2	1PPI:A:HIS305:ND1	2.072
						1PPI:A:GLN63:HE22	1.641
7d	-8.18	-0.24	1.00	-10.20	2	1PPI:A:LYS200:H23	2.159
						1PPI:A: HIS305:HD1	2.082
7e	-8.06	-0.22	1.24	-10.86	2	1PPI:A: HIS305:HD1	2.147
						1PPI:A:GLY306:HN	2.012
7f	-7.91	-0.26	1.58	-10.41	2	1PPI:A: HIS305:HD1	2.116
						1PPI:A:GLY306:HN	1.781
9a	-7.13	-0.15	5.98	-10.83	-	-	-
9b	-8.60	-0.23	499.43	-11.23	1	1PPI:A: ASP300:OD1	1.995
9c	-7.35	-0.23	4.10	-9.40	-	-	-
9d	-8.68	-0.26	430.94	-10.76	-	-	-
9e	-8.01	-0.24	1.34	-10.08	-	-	-
9f	7.24	-0.21	4.93	-9.73	2	1PPI:A: HIS305:HD1	1.872
						1PPI:A:GLY306:HN	2.163