Journal of Taibah University Medical Sciences (2014) 9(4), 293–300

Taibah University

Journal of Taibah University Medical Sciences

www.sciencedirect.com

Original Article

Synthesis, characterization and pharmacological studies of sulphur containing 1,2,4-triazole derivatives

D.V. Narayana Rao, Ph.D. [a], A. Raghavendra Guru Prasad, Ph.D. [b,*], Y.N. Spoorthy, Ph.D. [d], D. Raghunatha Rao, Ph.D. [c] and L.K. Ravindranath, Ph.D. [a]

[a] *Department of Chemistry, Sri Krishnadevaraya University, Anantapur, A.P., India*
[b] *IcfaiTech, Faculty of Science and Technology, ICFAI Foundation for Higher Education, Hyderabad, Telangana, India*
[c] *Extension and Training Division, National Institute of Nutrition, Indian Council for Medical Research, Hyderabad, Telangana, India*
[d] *Srikrishnadevaraya University, Anantapur, A.P., India*

Received 10 April 2014; revised 30 May 2014; accepted 7 June 2014; Available online 29 September 2014

الملخص

أهدف البحث: تم تصميم طريقة مكونة من ٥ خطوات لتحضير سبعة مركبات من مشتقات ١,٢,٤-الترايازول تحتوي على الكبريت كمركبات ابتدائية والكشف عن نشاطهم الدوائي.

طرق البحث: تم توصيف هذه المركبات عن طريق تحليل العناصر والبيانات الطيفية الجماعية. وتم تقييم جميع هذه المركبات لنشاطها المضاد للميكروبات تجاه عينات مختارة من البكتيريا والفطريات حسب الطرق المذكورة في الأبحاث السابقة. وتم تقييم الخصائص شبه الدوائية من خلال دراسات سيليكو.

النتائج: المركبات ٨أ، ٨ب، ٨ج أظهرت نشاطا متوسطا لمضادات الميكروبات. أما مركبات ٨د، ٨هـ، ٨و، ٨ز والمسمية مشتقات نيترو، كلورو، برومو، والفلورو على التوالي، أظهرت نشاطا أفضل كمضادات للميكروبات مقارنة بالمركبات الأخرى. أوضحت دراسات السيليكو أن مركب ٨ هـ مع الكلورو يمتلك خصائص شبه دوائية ممتازة مقارنة بالمركبات الأخرى قيد الدراسة.

الاستنتاجات: أظهرت جميع المركبات نشاطا جيدا مضادا للبكتيريا والفطريات. كما كشفت دراسات الفحص الظاهري أن المركبات قيد الدراسة تمتلك خصائص شبه دوائية ممتازة.

الكلمات المفتاحية: تصنيع; مشتقات ١,٢,٤-الترايازول; التحليل الطيفي; نشاط مضادات الميكروبات; خصائص شبه دوائية

Abstract

Objectives: To design a five step procedure for the synthesis of seven novel sulphur containing 1,2,4-triazole derivatives namely 4-[(3-(4-Chloro-phenoxymethyl)-5-(4-substituted-benzylsulfonyl)-1,2,4-triazol-4-yl)methyl]-morpholine from 4-Chloro-phenol and Ethyl-bromoacetate as starting compounds and to screen for their pharmacological activity.

Methods: The compounds were characterised by elemental analysis, IR, ^{1}H NMR and mass spectral data. All compounds were evaluated for antimicrobial activity against selected bacteria and fungi by the methods reported in the literature. The drug-like characteristics were assessed by *in silico* studies.

Results: The compounds 8a, b and c showed moderate antimicrobial activity. Compounds 8d, e, f and g namely nitro, chloro, bromo and fluoro derivatives respectively, showed better antimicrobial activity than the other compounds. *In silico* studies indicated that the compound 8e with chloro substituent possesses excellent drug-like characteristics among the compounds under study.

Conclusion: All the title compounds showed good antibacterial and antifungal activities. Virtual screening studies reveal that the compounds under study possess excellent drug-like characteristics.

Keywords: Synthesis; 1,2,4-triazole derivatives; Spectral analysis; Antimicrobial activity; Drug-like characteristics

* Corresponding address. Academic Coordinator, IcfaiTech, Faculty of Science and Technology, ICFAI Foundation for Higher Education, Hyderabad, Telangana, India.
E-mail: guruprasadar@yahoo.co.in (A. Raghavendra Guru Prasad)

Peer review under responsibility of Taibah University.

ELSEVIER Production and hosting by Elsevier

1658-3612 © 2014 Taibah University.
Production and hosting by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jtumed.2014.06.002

© 2014 Taibah University.
Production and hosting by Elsevier Ltd. All rights reserved.

Introduction

In the last few years, 1,2,4-triazoles and their fused heterocyclic derivatives have received considerable attention owing to their medicinal significance. Several drugs containing 1,2,4-triazole group i.e. Etizolam,[1] Alprazolam,[2] Furacylin[3] etc are well known. Particularly, diverse biological activities, such as antibacterial,[4] antifungal,[5] anti-inflammatory,[6] antituberculosis,[7] anticancer,[8] antioxidant[9] and InhA inhibitory activity[10] etc. have been associated with 1,2,4-triazole derivatives. Keeping in view the above mentioned facts and the medicinal importance of sulphur containing 1,2,4-triazole ring systems,[11–14] the authors have made an attempt to synthesize, characterize and evaluate the biological activity of some sulphur containing 1,2,4-triazoles. A number of pharmacologically active compounds have been reported[15–17] from these laboratories.

Materials and Methods

All Chemicals and reagents were procured from Ranbaxy Laboratories Ltd, Chemical Division, India. The standard bacterial and fungal strains were procured from National Centre for Cell Sciences, Pune, India. Nutrient broth, nutrient agar and 5 mm diameter antibiotic assay discs were obtained from Hi-Media Laboratories Limited, India. Melting points were determined by Scientific melting point apparatus, India and uncorrected. Synthesized compounds were recrystallized using suitable solvent. Digital electronics balance (Shankar Scientific Supplies, India), horizontal laminar air flow bench (Yorco Sales Pvt. Ltd, New Delhi, India), incubator (Yorco Sales Pvt. Ltd, New Delhi, India), zone reader (Cintex Industrial Corporation, India), hot air oven, autoclave and UV-Visible spectrophotometer (Shimadzu Corporation, Japan) were used for respective investigations. Elemental analysis was carried out on CHNS/O Elemental Analyser manufactured by PerkinElmer. The amount of halogens present in the compound was determined by the procedure reported in the literature.[18] Infrared spectra of the compounds were recorded in KBr discs on Perkin-Elmer FT-IR spectrometer (υ_{max} in cm^{-1}). ^1H NMR spectra were recorded on a JOEL (300 MHz) spectrometer using TMS as an internal standard (chemical shifts in δ). The mass spectra were recorded on a mass spectrometer JOEL sx-102.

Experimental section

Synthesis of ethyl-2-(4-chlorophenoxy)acetate (2)

4-Chloro-phenol (5 g, 29 mmol, 1.0 eq.) was added to a stirred suspension of Sodium hydride (1.12 g, 46.8 mmol, 1.2 eq) in DMF (25 mL) and the reaction mixture was stirred for 30 min. Ethylbromoacetate (9.8 g, 58 mmol, 1.5 eq) was added drop wise and was stirred for 3 h. The reaction mixture was poured in cold water, extracted with Ethyl acetate, organic layer was washed with water, brine solution, dried over anhydrous Sodium sulphate and the solvent was removed under reduced pressure to get crude compound. The crude solid was purified by silica gel (100−200 mesh) column chromatography, eluted with 2% Ethylacetate/Petroleum ether to get pure Ethyl-2-(4-chlorophenoxy)acetate (Yield:76%).

Synthesis of 2-(4-Chlorophenoxy)acetohydrazide (3)

A mixture of Hydrazine hydrate (0.980 g, 19.6 mmol, 4 eq.), Ethyl-2-(4-chlorophenoxy)acetate (2) (2.1 g, 9.8 mmol,1 eq.) in Ethanol (20 mL) was refluxed for 12 h. The reaction mixture was cooled to room temperature, filtered, so obtained solid was washed with Ethanol and dried under vacuum to get pure 2-(4-Chlorophenoxy)acetohydrazide (Yield:71%).

Synthesis of 1-(2-(4-Chlorophenoxy)acetyl) thiosemicarbazide (4)

Potassium thio cyanate (10.7 g, 110 mmol, 3.5 eq.) was added to a stirred solution of 2-(4-Chlorophenoxy)acetohydrazide (3) (6.2 g, 31 mmol,1 eq) in H_2O (31 mL) and HCl (7.75 mL) and the reaction mixture was heated to 90 °C for 4 h. The reaction mixture was cooled to room temperature, diluted with water and filtered. The solid so obtained was dried under vacuum to get crude 1-(2-(4-Chlorophenoxy) acetyl) thiosemicarbazide (Yield:79%).

Synthesis of 5-(4-Chlorophenoxymethyl)-2,4-dihydro-1,2,4-triazole-3-thione (5)

1-(2-(4-Chlorophenoxy)acetyl) thiosemicarbazide (4) (0.500 g, 1.92 mmol) was dissolved in saturated K_2CO_3 (70.0 mL) solution and stirred at room temperature for 2 days. The reaction mixture was filtered and the filtrate was acidified with 2N HCl. The reaction mixture was filtered and solid so obtained was dried under vacuum to get pure 5-(4-Chlorophenoxymethyl)-2,4-dihydro-1,2,4-triazole-3-thione (Yield:69%).

Synthesis of 3-Benzylsulfanyl-5-(4-chloro-phenoxymethyl)-4H-1,2,4-triazole (6a)

To a stirred solution of Potassium hydroxide in Ethanol (0.300 g in 15 mL), 5-(4-Chlorophenoxymethyl)-2,4-dihydro-1,2,4-triazole-3-thione (5) (0.483 g, 2 mmol) and Benzyl chloride (12 mL) were added. The reaction mixture was heated to reflux temperature for 4 h. The reaction mixture was cooled to room temperature, diluted with water and extracted with Ethylacetate. The organic layer was washed with water, brine solution, dried over anhydrous Sodium sulphate and the solvent was removed under reduced pressure to obtain 3-Benzylsulfanyl-5-(4-chloro-phenoxymethyl)-4*H*-1,2,4-triazole (6a). The product was isolated by recrystallization from a mixture of Ethylacetate-petroleum ether (1:1) (Yield:70%).

Synthesis of 4-[3-Benzylsulfanyl-5-(4-chloro-phenoxymethyl)-1,2,4-triazole-4-ylmethyl]-morpholine (7a)

A mixture of 3-Benzylsulfanyl-5-(4-chloro-phenoxymethyl)-4*H*-1,2,4-triazole (6a), (0.650 g, 1.6 mmol), Morpholine (6 mL) and water (15 mL) was stirred to obtain a clear solution. To this solution Formaldehyde (10 mL) and Dimethyl formamide (6 mL) were added, stirred for 2 h in ice-bath and left overnight at room temperature. White solid was isolated and recrystallized from Ethanol to give 4-[3-Benzylsulfanyl-5-(4-chloro-phenoxymethyl)-1,2,4-triazole-4-ylmethyl]-morpholine (7a) (Yield:71%). The procedure leading to the synthesis of 7a was extended to the synthesis of 7b-g.

Synthesis of 4-[(3-(4-Chloro-phenoxymethyl)-5-benzylsulfonyl)-1,2,4-triazol-4-yl)methyl]-morpholine 8a

To a solution of 4-[3-Benzylsulfanyl-5-(4-chloro-phenoxymethyl)-1,2,4-triazole-4-ylmethyl]-morpholine (7a) (0.700 g, 1.5 mmol) in Glacial acetic acid (5 mL), 30% Hydrogen peroxide (6.5 mL) was added and refluxed for about 2 h. The reaction mixture was cooled, filtered and solid obtained was recrystallized from Ethanol solution. The procedure leading to formation of 8a was extended for the synthesis of 8b–8g. The structure of 8a–8g was confirmed by elemental analysis, IR, ^{1}H NMR and mass spectral data. The reaction scheme is shown in the Scheme 1.

Antimicrobial activity

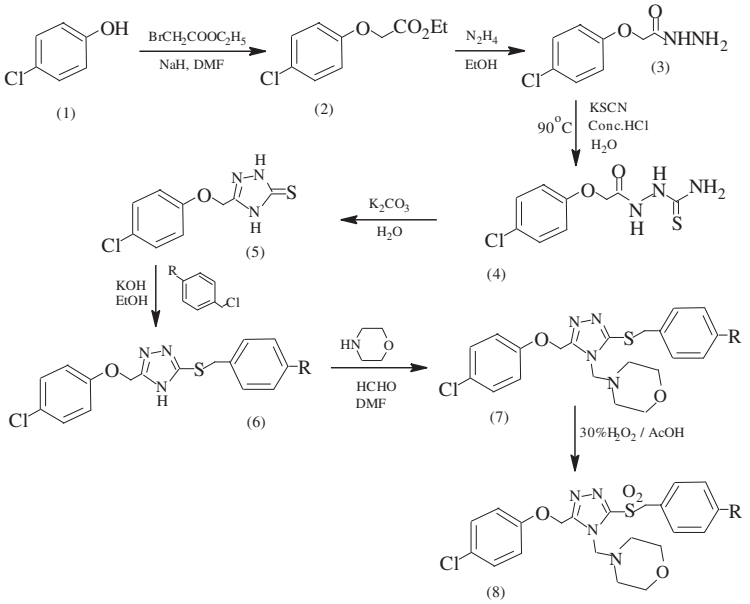
Disc diffusion method

A suspension of *Staphylococcus aureus* was added to sterile nutrient agar at 45 °C. The mixture was transferred to sterile petridishes to give a depth of 3 to 4 mm and allowed to solidify. Sterile discs of 5 mm diameter (made from Whatmann Filter paper) were immersed in solutions of synthesized compounds (50 µg/ml) and untreated control sample was also prepared for comparison.

A period of pre incubation diffusion (1 h at room temperature) was ensured to minimize the effects of variations in time. The plates were incubated at 37 °C for 24 h and observed for antibacterial activity. The diameter of zone of inhibition was measured for each plate in which the zone of inhibition was observed. The average zone of inhibition was calculated and compared with that of the standard. Similar procedure was adopted for studying the antimicrobial activity against the other organisms.

Broth dilution method

Minimum Inhibitor Concentration was found out by broth dilution method. Standardized Inoculum (matched to McFarland $BaSO_4$ standard) of suspension of organisms were prepared. A series of glass tubes containing different concentrations of test compounds dissolved in Dimethylsuphoxide and spiller in nutrient broth were incubated with one drop of inoculum and mixed gently by shaking the rack. Two growth control tubes were also prepared without the addition of test compound and its optical density was determined. The tubes were incubated for 24 hours at 37 °C in air. The turbidity produced in each tube was recorded by UV-Visible spectrophotometer. The turbidity produced by the broth (without inoculum) was considered as 100% transparency. Minimum inhibitory concentration (MIC) was noted as the concentration of the test substance, which completely inhibits the growth of the microorganism i.e. 100% transparency.



Compound	8a	8b	8c	8d	8e	8f	8g
R	H	CH_3	OCH_3	NO_2	Cl	Br	F

Scheme 1: Synthesis of 4-[(3-(4-Chloro-phenoxymethyl)-5-(4-substituted-benzylsulfonyl)-1,2,4-triazol-4-yl)methyl]-morpholine 8.

The antibacterial activity was compared with that of Cefaclor and the antifungal activity was compared with that of Ketoconazole.

Results

The novel compounds synthesised were characterised by elemental analysis, IR and, ^{1}H NMR and mass spectral data. Elemental analysis details of 4-[(3-(4-Chloro-phenoxymethyl)-5-(4-substituted-benzylsulfonyl)-1,2,4-triazol-4-yl) methyl]-morpholine 8 are given in the Table 1.

The characterisation details are given below.

IR spectral data of Ethyl-2-(4-Chlorophenoxy)acetate (2)

Aromatic Ar-H (3058 cm^{-1}), aliphatic-CH_2-(2923 cm^{-1}) and >C=O of −COOEt (1656 cm^{-1}).

^{1}H NMR spectral data of Ethyl-2-(4-Chlorophenoxy)acetate (2)

δ.7.21 (d, 2H, Ar-H); 7.1 (d, 2H, Ar-H); 4.9 (s, 2H, -O-CH_2-); 4.0 (q, 2H, -CH_2 of ethyl group); 1.21 (t, 3H, CH_3 of ethyl group).

IR spectral data of 2-(4-Chlorophenoxy)acetohydrazide (3)

NH_2 (3425 cm^{-1}); NH (3125 cm^{-1}); Aromatic Ar-H (3060 cm^{-1}); aliphatic -CH_2-, (2928 cm^{-1}) and >C=O of −CO-NH- (1645 cm^{-1}).

^{1}H NMR spectral data of 2-(4-Chlorophenoxy)acetohydrazide (3)

δ 2.80 (broad s, 2H, -NH_2); 5.20 (s, 2H, -O-CH_2-); 7.00 (d, 2H, Ar-H); 7.20 (d, 2H, Ar-H); 9.1 (broad s, 1H, -CO-NH-).

IR spectral data of 1-(2-(4-Chlorophenoxy)acetyl)thiosemicarbazide (4)

NH_2 (3420 cm^{-1}); NH (3130 cm^{-1}); Aromatic Ar-H (3064 cm^{-1}); aliphatic -CH_2- (2930 cm^{-1}); >C=O of −CO-NH- (1648 cm^{-1}); N-N (1550 cm^{-1}) and C=S (1134 cm^{-1}).

^{1}H NMR spectral data of 1-(2-(4-Chlorophenoxy)acetyl) thiosemicarbazide (4)

δ 2.70 (broad s, 2H, -NH_2); 5.10 (s, 2H, -O-CH_2-); 7.10 (d, 2H, Ar-H); 7.20 (d, 2H, Ar-H); 9.15 (broad s, 1H, -CO-NH-).

IR spectral data of 5-(4-Chlorophenoxymethyl)-2,4-dihydro-1,2,4-triazole-3-thione (5)

NH (3136 cm^{-1}); Aromatic Ar-H (3066 cm^{-1}); aliphatic -CH_2- (2932 cm^{-1}); >C=N, (1597 cm^{-1}); N-N (1615 cm^{-1}) and C=S (1143 cm^{-1}).

^{1}H NMR spectral data of5-(4-Chlorophenoxymethyl)-2,4-dihydro-1,2,4-triazole-3-thione (5)

δ 5.10 (s, 2H, -O-CH_2-); 6.80 (d, 1H, Ar-H); 7.10 (s, 1H, Ar-H); 7.20 (s, 2H, Ar-H); 7.40 (d, 2H, Ar-H); 8.15 (broad s, 1H, -C=S-NH-); 14.70 (broad signal due to thio-thione tautomeric form).

IR spectral data of 3-Benzylsulfanyl-5-(4-chloro-phenoxymethyl)-4H-1,2,4-triazole (6a)

NH (3140 cm^{-1}); Aromatic Ar-H (3068 cm^{-1}); aliphatic -CH_2- (2934 cm^{-1}); >C=N (1599 cm^{-1}) and N-N (1640 cm^{-1}).

^{1}H NMR spectral data of 3-Benzylsulfanyl-5-(4-chlorophenoxymethyl)-4H-1,2,4-triazole (6a)

δ 4.90 (s, 2H, -S-CH_2-); 5.30 (s, 2H, -O-CH_2-); 6.66 (d, 2H, Ar-H); 6.90 (d, 2H, Ar-H); 7.28−7.38 (m, 5H, C_5H_5 attached to S-CH_2-); 13.80 (broad signal due to thiol-thione tautomeric form).

IR spectral data of 4-[3-Benzylsulfanyl-5-(4-chloro-phenoxymethyl)-1,2,4-triazole-4-ylmethyl]-morpholine (7a)

NH (3143 cm^{-1}); Aromatic Ar-H (3071 cm^{-1}); aliphatic -CH_2 (2936 cm^{-1}); >C=N (1598 cm^{-1}) and N-N (1655 cm^{-1}).

^{1}H NMR spectral data of 4-[3-Benzylsulfanyl-5-(4-chlorophenoxymethyl)-1,2,4-triazole-4-ylmethyl]-morpholine (7a)

δ 2.45 (m, 4H, morpholine N-CH_2); 3.63 (m, 4H, morpholine O-CH_2-); 4.63 (s, 2H, >CH_2); 5.20 (s, 2H, -S-CH_2-); 5.80 (s, 2H, -O-CH_2-); 6.69 (d, 2H, Ar-H); 6.94 (d, 2H, Ar-H); 7.36−7.44 (m, 5H, C_5H_5 attached to S-CH_2-).

Spectral data of 4-[(3-(4-Chloro-phenoxymethyl)-5-(4-substituted-benzylsulfonyl)-1,2,4-triazol-4-yl)methyl]-morpholine 8 (IR-ν_{max} in cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm.

IR spectral data of 8a: 1028 (C−S); 1518 (C=N);1613 (N-N).

^{1}H NMR spectral data of 8a: 2.48-2.52 (m, 4H, morpholine N-CH_2); 3.60−3.70 (m, 4H, morpholine O-CH_2-); 4.68 (s, 2H, >CH_2); 4.89 (s, 2H, -SO_2-CH_2); 5.23 (s, 2H, -O-CH_2-); 6.50 (d, 2H, Ar-H); 6.88 (d, 2H, Ar-H); 7.01−7.19 (m, 5H, C_5H_5 attached to S-CH_2-);

IR spectral data of 8b: 1030 (C−S); 1520 (C=N); 1616 (N-N).

^{1}H NMR spectral data of 8b: 2.30−2.60 (m, 4H, morpholine N-CH_2); 2.80 (s, 3H, -CH_3); 3.80−3.90 (m, 4H, morpholine O-CH_2-); 4.60 (s, 2H, >CH_2); 4.83 (s, 2H, -SO_2-CH_2); 5.13 (s, 2H, -O-CH_2-); 6.42 (d, 2H, Ar-H); 6.84 (d, 2H, Ar-H); 7.03 (d, 2H, Ar-H); 7.13 (d, 2H, Ar-H).

IR spectral data of 8c: 1032 (C−S); 1523 (C=N); 1628 (N-N).

^{1}H NMR spectral data of 8c: 2.52−2.56 (m, 4H, morpholine N-CH_2); 3.90−4.00 (m, 4H, morpholine O-CH_2-);

Table 1: Characterization data of 4-[(3-(4-Chloro-phenoxymethyl)-5-(4-substituted-benzylsulfonyl)-1,2,4-triazol-4-yl)methyl]-morpholine 8.

Compd.	Molecular formula	Yield (%) m. p(°C)	Analysis (%) found (calculated)					
			C	H	N	O	S	Cl
8a	$C_{21}H_{23}ClN_4O_4S$	51,156−159	54.32 (54.48)	4.88 (5.01)	12.45 (12.10)	13.62 (13.82)	6.97 (6.93)	7.83 (7.66)
8b	$C_{22}H_{25}ClN_4O_4S$	53,167−170	55.04 (55.40)	5.37 (5.28)	11.92 (11.75)	13.65 (13.42)	6.85 (6.72)	7.67 (7.43)
8c	$C_{22}H_{25}ClN_4O_5S$	47,147−150	53.84 (53.60)	5.29 (5.11)	11.67 (11.37)	16.65 (16.23)	6.78 (6.50)	7.35 (7.19)
8d	$C_{21}H_{23}ClN_5O_6S$	48,164−168	48.91 (49.66)	4.21 (4.37)	13.70 (13.79)	19.42 (18.90)	6.58 (6.31)	7.22 (6.98)
8e	$C_{21}H_{23}Cl_2N_4O_4S$	50,173−175	51.05 (50.71)	4.29 (4.46)	11.55 (11.26)	13.12 (12.87)	6.23 (6.45)	14.02 (14.26)
8f	$C_{21}H_{22}BrClN_4O_4S$	52,169−171	46.41 (46.55)	4.56 (4.09)	9.79 (10.34)	12.12 (11.81)	6.34 (5.92)	6.12 (6.54)
8g	$C_{21}H_{22}FClN_4O_4S$	50,175−179	52.53 (52.44)	4.43 (4.61)	11.21 (11.65)	13.49 (13.31)	6.98 (6.67)	7.56 (7.37)

3.40 (s, 3H, -CH_3); 4.69 (s, 2H, >CH_2); 4.90 (s, 2H, -SO_2-CH_2-); 5.25 (s, 2H, -O-CH_2-); 6.53 (d, 2H, Ar-H); 6.88 (d, 2H, Ar-H); 6.90 (d, 2H, Ar-H); 7.19 (d, 2H, Ar-H).

IR spectral data of 8d: 1038 (C—S); 1530 (C═N); 1625 (N-N).

^{1}H NMR spectral data of 8d: 2.58—2.61 (m, 4H, morpholine N-CH_2); 4.00—4.20 (m , 4H, morpholine O-CH_2-); 4.70 (s, 2H, >CH_2); 4.91 (s, 2H, -SO_2-CH_2-); 5.28 (s, 2H, -O-CH_2-); 6.58 (d, 2H, Ar-H); 6.86 (d, 2H, Ar-H); 7.15 (d, 2H, Ar-H); 7.90 (d,2H, Ar-H);

IR spectral data of 8e: 1029 (C—S); 1522 (C═N); 1616 (N-N).

^{1}H NMR spectral data of 8e: 2.60—2.64 (m, 4H, morpholine N-CH_2); 3.80—3.90 (m, 4H, morpholine O-CH_2-); 4.71 (s, 2H, >CH_2); 4.92 (s, 2H, -SO_2-CH_2-); 5.29 (s, 2H, -O-CH_2-); 6.60 (d, 2H, Ar-H); 6.87 (d, 2H, Ar-H); 7.10 (d, 2H, Ar-H); 7.43 (d,2H, Ar-H);

IR spectral data of 8f: 1027 (C—S); 1524 (C═N); 1617 (N-N).

^{1}H NMR spectral data of 8f: 2.54—58 (m, 4H, morpholine N-CH_2); 3.50—3.60 (m, 4H, morpholine O-CH_2-); 4.65 (s, 2H, >CH_2); 4.85 (s, 2H, -SO_2-CH_2-); 5.26 (s, 2H, -O-CH_2-); 6.54 (d, 2H, Ar-H); 6.85 (d, 2H, Ar-H); 7.12 (d, 2H, Ar-H); 7.40 (d, 2H, Ar-H);

IR spectral data of 8g: 1028 (C—S); 1526 (C═N); 1619 (N-N).

^{1}H NMR spectral data of 8g: 2.62—65 (m, 4H, morpholine N-CH_2); 4.00—4.20 (m, 4H, morpholine O-CH_2-); 4.71 (s, 2H, >CH_2); 4.93 (s, 2H, -SO_2-CH_2-); 5.30 (s, 2H, -O-CH_2-); 6.62 (d, 2H, Ar-H); 6.89 (d, 2H, Ar-H); 7.12 (d, 2H, Ar-H); 6.90 (d, 2H, Ar-H):

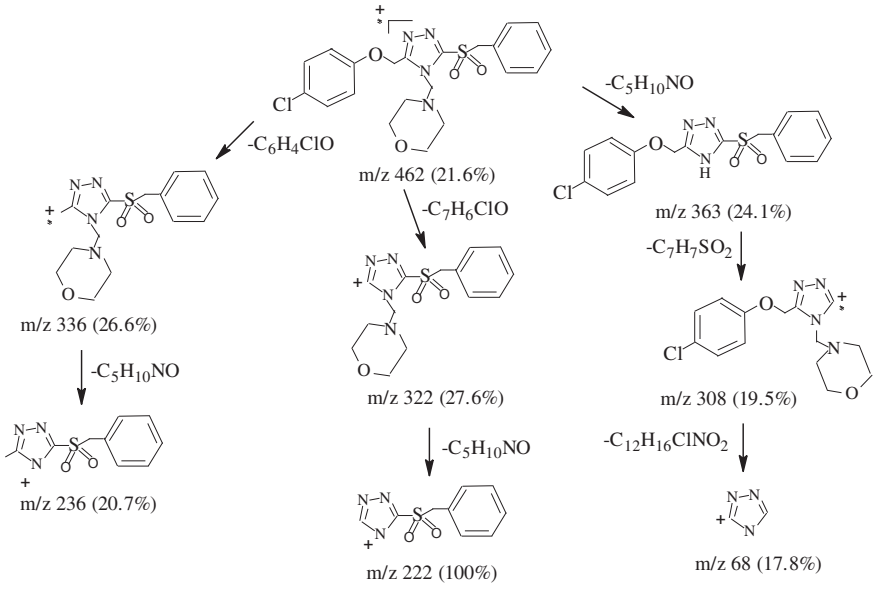
Discussion

2-(4-Chlorophenoxy)acetohydrazide (3) was obtained by the treatment of Hydrazine hydrate with Ethyl-2-(4-chlorophenoxy)acetate (2) in Ethanol medium. An aqueous solution of 3 on treatment with Potassium thiocyanate in presence of HCl resulted in the formation of 1-(2-(4-Chlorophenoxy) acetyl) thiosemicarbazide (4) which on further treatment with an aqueous solution of K_2CO_3 yielded 5-(4-Chlorophenoxymethyl)-2,4-dihydro-1,2,4-triazole-3-thione (5). 5 on treatment with *p*-substituted Benzyl chloride in an Ethanolic solution of KOH resulted in the formation of corresponding triazole 6. The corresponding Mannich product of 7 series was obtained by the reaction of 6 with an aqueous solution of Morpholine which further was converted into sulfonyl derivative (8a-g). The formation of products at each step was confirmed by elemental and spectral analysis. Mass spectral fragmentation of 4-[(3-(4-Chloro-phenoxymethyl)-5-benzylsulfonyl-1,2,4-triazol-4-yl)methyl]-morpholine 8a is presented in Scheme 2. The molecular ion peak was observed at m/z 462(21.6%), the base peak was at m/z 222(100%), other prominent peaks were appeared at m/z 68(17.8%), 236(20.7%), 322(27.6%), 308(19.5%) and 363(24.1%).

Antimicrobial activity

The preliminary antimicrobial activity of synthesized compounds was investigated by disc diffusion method[19] against the following pathogenic organisms. The gram positive bacteria screened were *Staphylococcus aureus* NCCS 2079 and *Bacillus cereus* NCCS 2106. The gram negative bacterial screened were *Escherichia coli* NCCS 265 and *Pseudomonas aeruginosa* NCCS2200. The fungi screened were *Aspergillus niger* NCCS 1196 and *Candida albicans* NCCS 3471. Minimum inhibitory concentration was found by broth dilution method.[20]

The results (Tables 2 and 3) showed that all the novel compounds were active against the tested microbes.



Scheme 2: Mass spectral fragmentation details of 4-[(3-(4-Chloro-phenoxymethyl)-5-benzylsulfonyl-1,2,4-triazol-4-yl)methyl]-morpholine 8a.

Table 2: Antimicrobial activity of 4-[(3-(4-Chloro-phenoxymethyl)-5-(4-substituted-benzylsulfonyl)-1,2,4-triazol-4-yl)methyl]-morpholine 8.

Compound (50 mg/mL)	Zone inhibition (mm)[a]					
	Staphylococus aureus NCCS 2079	*Bacillus Cereus* NCCS 2106	*Escherichia coli* NCCS 2065	*Pseudomanas aeruginos* NCCS 2200	*Aspergillus niger* NCCS 1196	*Candida albicans* NCCS 2106
8a	7.17	4.67	7.33	4.83	5.83	7.67
8b	6.83	5.17	6.33	6.5	6.83	7.00
8c	6.00	3.33	5.67	6.00	5.83	7.33
8d	15.17	13.83	12.17	12.00	16.00	16.17
8e	14.00	12.17	10.83	11.00	14.83	14.50
8f	12.00	10.83	8.50	7.50	14.17	11.50
8g	13.17	11.33	9.17	9.00	14.83	12.33
Cefaclor (10 mg/mL)	19.83	22.00	20.00	20.17	–	–
Ketoconazole (25 mg/mL)	–	–	–	–	22.17	24.83

[a] Average of six determinations.

However none of them demonstrated superior activity to that of standards tested. Compound '8d' i.e. containing nitro group at 4 position exhibited highest antimicrobial activity among the title compounds followed by compounds '8 e, g and f' containing halogen group at 4 position. The antimicrobial activity of compounds under investigation is in the order d > e > g > f > a > b > c.

Octanol-water partition coefficient is expressed in terms of milogP and is a measure of molecular hydrophobicity in rational drug design. All the values are found to be well within the accepted range.[21] As per the computationally predicted characteristics, their pharmacological activity is of the order e > g > c > a > b > d > f. The compound 8e with chloro substituent is predicted to possess excellent drug-like characteristics among the compounds under study. In the case of compounds 8d and f, Table 4 indicates violations as a result of higher molecular weight and higher number of donor atoms.[22] However, it is frequently necessary to work with compounds of high molecular weight to achieve preferred drug features. Daniel et al.[23] suggests that success in achieving high oral bioavailability depends up on molecular rigidity and polar surface area without specific reference to molecular weight. Though high molecular weight, non bonded intramolecular interactions lead to reduced flexibility in large cyclic molecules, it was reported that[23] reported that efficient selective criteria for oral bioavailability is a result of precise combination of factors such as polar surface area (PSA) $\leq$ 140 Å, number of rotatable bonds $\leq$10 and sum of H-bond donors and acceptors $\leq$12 without specific limit to molecular weight.

Conclusion

All 1,2,4-triazole derivatives reported showed good antibacterial and antifungal activities. The preliminary antimicrobial activity studies were done by disc diffusion method and minimum inhibitory concentration was found out by broth dilution method. Compounds 8a, b and c showed moderate antimicrobial activity. Compounds 8d, e, f and g namely nitro, chloro, bromo and fluoro derivatives respectively, showed better antimicrobial activity than the other compounds. Virtual screening studies revealed that the

Table 3: Minimum inhibitory concentration of 4-[(3-(4-Chloro-phenoxymethyl)-5-(4-substituted-benzylsulfonyl)-1,2,4-triazol-4-yl) methyl]-morpholine 8.

Compound	Minimum inhibitory concentration (μg/mL) ± SD[a]					
	Staphylococus aureus NCCS 2079	*Bacillus Cereus* NCCS 2106	*Escherichia coli* NCCS 2065	*Pseudomanas aeruginos* NCCS 2200	*Aspergillus niger* NCCS 1196	*Candida albicans* NCCS 2106
8a	15.24 ± 0.08	18.18 ± 0.07	14.41 ± 0.09	19.49 ± 0.07	21.62 ± 0.18	17.47 ± 0.22
8b	16.12 ± 0.11	17.74 ± 0.06	15.05 ± 0.07	15.62 ± 0.04	16.17 ± 0.29	16.28 ± 0.26
8c	19.29 ± 0.14	21.36 ± 0.16	18.25 ± 0.09	18.43 ± 0.12	19.30 ± 0.23	17.39 ± 0.23
8d	5.17 ± 0.11	6.32 ± 0.13	7.08 ± 0.13	8.13 ± 0.13	5.30 ± 0.24	5.44 ± 0.23
8e	6.08 ± 0.16	6.85 ± 0.12	7.10 ± 0.14	8.19 ± 0.07	5.71 ± 0.22	5.66 ± 0.23
8f	8.19 ± 0.13	7.84 ± 0.11	10.07 ± 0.14	9.51 ± 0.15	6.57 ± 0.27	7.37 ± 0.20
8g	6.63 ± 0.18	7.08 ± 0.17	8.34 ± 0.11	7.29 ± 0.21	6.40 ± 0.21	7.19 ± 0.25
Cefaclor	2.08 ± 0.15	4.04 ± 0.13	3.02 ± 0.15	3.00 ± 0.16	–	–
Ketoconazole	–	–	–	–	0.75 ± 0.15	0.47 ± 0.13

Pharmacophore analysis.

Drug like properties mentioned in Table 4 is predicted from Molinspiration Cheminformatics 2014 and admet SAR.

[a] n = 6; SD = Standard deviation.

Table 4: Pharmacophore analysis of title compounds.

	Pharmacophore Characteristic	8a	8b	8c	8d	8e	8f	8g
[a]Toxicity	AMES toxicity (all are classified as non AMES toxic)	0.5706	0.5726	0.5688	0.5000	0.5702	0.5669	0.5706
	Carcinogens (all are classified as non-carcinogens)	0.6274	0.6028	0.6423	0.5823	0.6248	0.6327	0.6274
[b]Properties	miLogP	3.145	3.594	3.202	3.104	3.309	3.954	3.823
	TPSA	86.566	86.566	95.8	132.39	86.566	86.566	86.566
	natoms	31	32	33	34	32	32	32
	MW	462.959	476.986	492.985	507.956	480.949	541.856	497.404
	nON	8	8	9	11	8	8	8
	nOHNH	0	0	0	0	0	0	0
	nviolations	0	0	0	2	0	1	0
	nrotb	8	8	9	9	8	8	8
	volume	386.406	402.967	411.952	409.74	391.338	404.292	399.942
	[a]Aqueous solubility (LogS)	−3.8442	−3.8442	−3.7324	−3.8394	−3.8411	−3.8422	−3.8442
	[a]$Caco^{-2}$ Permeability ($LogP_{app}$, cm/s)	0.6542	0.7196	0.6885	0.7388	0.6598	0.6499	0.6542
[b]Bioactivity	GPCR ligand	−0.35	−0.38	−0.36	−0.44	−0.35	−0.42	−0.34
	Ion channel modulator	−0.75	−0.78	−0.74	−0.73	−0.73	−0.79	−0.74
	Kinase inhibitor	−0.31	−0.33	−0.3	−0.39	−0.3	−0.34	−0.28
	Nuclear receptor ligand	−0.62	−0.62	−0.61	−0.64	−0.6	−0.69	−0.58
	Protease inhibitor	−0.45	−0.48	−0.45	−0.52	−0.43	−0.53	−0.46
	Enzyme inhibitor	−0.32	−0.36	−0.33	−0.38	−0.31	−0.37	−0.33

[a] Predicted using admet SAR.
[b] Molinspiration cheminformatics 2014.

compounds under study possess excellent drug-like characteristics.

Conflict of interests

The authors declare no competing interests relating to this article.

References

1. Shiroki M, Tahara T, Araki K. Jap patent, 75100096, (1975). **Chem Abstr 1976**; 84: 59588k.
2. Coffen DL, Fryer RI. US patent, 3, 849434, (1974). **Chem Abstr 1975**; 82: 730044v.
3. Povelista FE, Gural AG. Antibiotiki Moscow, 18, 71, (1973). **Chem Abstr 1973**; 78: 93044.
4. El-Sayed Ali Tarik, El-Kazak Azza Mohammed. Synthesis and antimicrobial activity of some new 1,3-thiazoles, 1,3,4-thiadiazoles, 1,2,4-triazoles and 1,3-thiazines incorporating acridine and 1,2,3,4-tetrahydroacridine moieties. **Eur J Chem 2010**; 1: 6–11.
5. Bai JK, Zhao W, Li HM, Tang YJ. Novel biotransformation process of podophyllotoxin to 4 β-sulfur-substituted podophyllum derivates with anti-tumor activity by penicillium purpurogenum. **Curr Med Chem 2012**; 19: 927–936.
6. Mathew V, Keshavayya J, Vaidya VP. Heterocyclic system containing bridgehead nitrogen atom: synthesis and pharmacological activities of some substituted 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles. **Eur J Med Chem 2006**; 41: 1048–1058.
7. Shiradkar MR, Kiran Kumar M, Gangadasu HR, Suresh T, Kalyan CA, Panchal D, Ranjit K, Prashan B, Jyothi G, Vinod M, Mayuresh T. Synthesis of new S-derivatives clubbed with triazolyl thiazoleas anti mycobacterium tuberculosis agent. **Bioorg Med Chem 2007**; 15: 3997–4008.
8. Sztanke K, Tomasz T, Jolanta R, Kazimierz P, Martyna K. Synthesis, determination of the lipophilicity, anticancer and antimicrobial properties of some fused 1,2,4 triazolederivatives. **Eur J Med Chem 2008**; 43: 404–419.
9. Khan I, Ali Shajid, Hameed Shahid, Rama Naseem Hasan, Hussain Muhammed Tahir, Wabood Abdul, Reazuddin, Ul-Haq Zaheer, Khan Ajmal, Iqbal MC. Synthesis, antioxidant activities and urease inhibition of some 1,2,4-triazole and 1,3,4-thiadiazole derivaties. **Eur J Med Chem 2010**; 45: 5200–5207.
10. Christophe M, Sylvain G, Christian L, Maria RP, Frederic F, Cyril I, Michel B. Synthesis and biological activity of triazole derivatives as inhibitors of InhA and anti tuberculosis agents. **Eur J Med Chem 2011**; 46: 5524–5531.
11. Holla BS, Veerendra B, Shivananda MK, Poojary B. Synthesis characterization and anticancer activity studies on some mannich bases derived from 1,2,4-triazoles. **Eur J Med Chem 2003**; 38: 759–767.
12. Mathew V, Keshavayya J, Vaidya VP, Giles D. Studies on synthesis and pharmacological activities of 3,6-disubstituted-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles and their dihydro analogues. **Eur J Med Chem 2007**; 42: 823–840.
13. Palaska E, Sahin G, Kelicen P, Durlu NT, Altınok G. Synthesis and anti-inflammatory activity of 1-acylthiosemi-carbazides, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazole-3-thiones. **Farmaco 2002**; 57: 101–107.
14. Dogan HN, Duran A, Rollas S. Synthesis and preliminary anticancer activity of new 1H-4,5-dihydro-3-(3-hydroxy-2-naphthyl)-4-substituted-1,2,4-triazoline-5-thiones. **Indian J Chem 2005**; 44B: 2301–2307.
15. Krishna Naik E, Raghavendra Guru Prasad A, Spoorthy YN, Ravindranath LK. Novel compounds containing 1,3,4-oxadiazole and pyrazole-3-one nuclei – synthesis and antimicrobial evaluation. **An R Acad Nac Farm 2014**; 80: 201–213.
16. Sreedevi M, Raghavendra Guru Prasad A, Spoorthy YN, Ravindranath LK. Synthesis and antimicrobial evaluation of certain novel thiazoles. **Adv Pharm Bull 2013**; 3: 227–230.
17. Narayana Rao DV, Raghavendra Guru Prasad A, Spoorthy YN, Pariplavi M, Ravindranath LK. Synthesis,

characterization and biological studies of substituted quinozoline-4-(3H)-one containing diazepine moiety. **Ann Pharm Fr 2014**; 72: 51–58.
18. Elek A, Hill DW. The micro estimation of halogens in organic compounds. **J Am Chem Soc 1933**; 55: 2550–2554.
19. Arthington-Skaggs BA, Motley M, Warnock DW, Morrison CJ. Comparative evaluation of PASCO and National Committee for Clinical Laboratory Standards M27-A broth micro dilution methods for antifungal drug susceptibility testing of yeasts. **J Clin Microbiol 2000**; 38: 2254–2260.
20. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. **Nat Protoc 2008**; 3: 163–175.
21. Shamsuzzaman A, Mashrai, Ahmad A, Dar AM, Khanam H, Danishuddin M, Khan AU. Synthesis, evaluation and docking studies on steroidal pyrazolones as anticancer and antimicrobial agents. **Med Chem Res 2014**; 23: 348–362.
22. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. **Adv Drug Deliv Rev 1997**; 23: 3–25.
23. Daniel FV, Stephen RJ, Cheng Hung-Yuan, Brian RS, Keith WW, Kenneth DK. Molecular properties that influence the oral bioavailability of drug candidates. **J Med Chem 2002**; 45: 2615–2626.