European Journal of Medicinal Chemistry 45 (2010) 3446–3452

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis, antibacterial activity evaluation and QSAR studies of novel dispiropyrrolidines

K. Karthikeyan [a], P.M. Sivakumar [b], M. Doble [b], P.T. Perumal [a,*]

[a] Organic Chemistry Division, Central Leather Research Institute, Adyar, Chennai-600 020, India
[b] Department of Biotechnology, Indian Institute of Technology Madras, Chennai-600 036, India

ARTICLE INFO

Article history:
Received 10 February 2010
Received in revised form
28 April 2010
Accepted 30 April 2010
Available online 6 May 2010

Keywords:
Azomethine ylide cycloaddition
Dispiropyrrolidine
Antibacterial activity
QSAR studies

ABSTRACT

A series of novel dispiropyrrolidines have been synthesized through 1,3-dipolar cycloaddition of an azomethine ylide generated from sarcosine and isatin with the dipolarophile 3-benzylidene-1-methyl-pyrrolidine-2,5-dione. Their antibacterial activity was evaluated against *Bacillus subtilis* NCIM 2718, *Staphylococcus aureus* NCIM5021, *Salmonella typhi* NCIM2501, *Pseudomonas aeruginosa* NCIM 5029 and *Proteus vulgaris* NCIM2813 by two fold dilution method. Compound **6e** exhibits reasonably good activity and compound **6c** exhibits poor activity against all the organisms. The QSAR's were developed for all antibacterial activities. The models had either one or two descriptors ($r^2 = 0.81–0.97$, $r^2_{adj} = 0.75–0.96$, $q^2 = 0.57–0.92$, *F*-ratio = 12.73–162.76). Topology, shape, charge distribution and hydrophobic nature of the molecules had pronounced effect on their antibacterial activity.

1. Introduction

1,3-Dipolar cycloaddition reactions is an efficient method for the construction of heterocyclic units in a highly regio- and stereoselective manner [1]. In particular, the chemistry of azomethine ylides have gained significance in recent years as it serves as an expedient precursor for the constructing nitrogen-containing five membered heterocycles, which constitute the central skeleton of numerous natural products [2]. The intermolecular [3 + 2]-cycloaddition reaction of azomethine ylides with various alkenes represents an efficient and convergent method for the synthesis of pyrrolidine and pyrrolizidine units. Functionalised pyrrolidine alkaloids constitute classes of compounds with significant biological activities [3]. The 1,3-dipole (azomethine ylide) generated by the decarboxylative condensation of isatin and secondary amino-acid reacts with dipolarophiles containing an exocyclic double bond to afford novel spiro pyrrolidine ring systems [4]. Spiro compounds represent an important class of naturally occurring substances characterized by their pronounced biological properties [5–11]. The spirooxindole ring system forms the core structure of many pharmacological agents and alkaloids. Spirooxindole derivatives are found to be potent aldose reductase inhibitors (ARIs), which help to treat and prevent diabetic complications arising from elevated levels of sorbitol [12] and behave as poliovirus and rhinovirus 3C-proteinase inhibitors [13].

Bacterial strains acquire resistance against commercial drugs due to their regular usage and pose a threat to human kind. Designing newer antibacterial agents to combat this resistance and in the treatment of bacterial infections has forced researchers to continuously search for new chemical entities with anti-infective properties. Hence our studies have been focused towards the synthesis and bio-evaluation of dispiropyrrolidine derivatives as possible drug candidates.

2. Chemistry

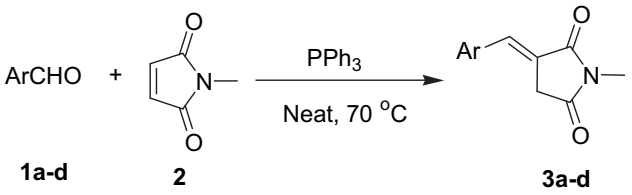
In continuation of our studies in the area of cycloaddition reactions [14] and with a view to synthesise of novel dispiroheterocyclic derivatives, we herein report the 1,3-dipolar cycloaddition reaction of 3-benzylidene-1-methyl-pyrrolidine-2,5-dione derivatives as 2π components with various azomethine ylides for the facile synthesis of biologically active dispiro pyrrolidines.

In this reaction we prepared 3-benzylidene-1-methyl-pyrrolidine-2,5-dione (dipolarophile) **3a**–**d** from *N*-methyl maleimide and various substituted benzaldehydes using PPh_3 under neat conditions at 70 °C (Scheme 1, Table 1).

The 1,3-dipolar cycloaddition of azomethine ylide generated *insitu* from the reaction of isatin **4a**–**d** and sarcosine **5** with 3-benzylidene-1-methyl-pyrrolidine-2,5-dione **3a**–**d** in methanol

* Corresponding author. Tel./fax: +91 44 24913289.
E-mail address: ptperumal@gmail.com (P.T. Perumal).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.04.035



Scheme 1. Synthesis of 3-benzylidene-1-methyl-pyrrolidine-2,5-dione **3**.

at reflux temperature yielded a series of dispiropyrrolidines **6a**–**i** in good yield (Scheme 2, Table 2). The reaction afforded a single product in all cases, as evidenced by thin layer chromatography (TLC).

The structure of the dispiropyrrolidine derivatives was confirmed by spectral analysis. IR spectrum of the pyrrolo-oxindole derivative **6d**, showed a peak at 1710 cm^{-1} due to the carbonyl group of the oxindole moiety. The ^1H NMR spectrum exhibited three triplet at δ 3.39 (J = 8.4 Hz), 3.74 (J = 9.2 Hz) and 4.15 (J = 9.2 Hz) were assigned to protons Ha, Hb and Hc of the pyrrolidine ring respectively. The ^{13}C NMR, two peaks at δ 61.3 and 77.7 ppm corresponds to the two spiro carbons and the amide carbonyl carbons resonated at δ 174.4, 175.6 and 179.5 ppm. The mass spectrum revealed the molecular ion peak ($M^+ + 1$) at m/z 420. The structure was further confirmed through X-ray diffraction studies [15] (Fig. 1).

3. Biology

Table 3 shows the observed antibacterial activities of dispiropyrrolidines given as micromolar concentration (μM) against five of the organisms evaluated by two fold dilution method. Compound **6e** is reasonably active against all the organisms and compounds **6b**, **6g** and **6h** are also active against majority of the organisms and they have 2-OH or 4-Cl substitution in the aromatic ring of the dipolarophile. Compound **6c**, **6d** and **6f** with a 4-OCH_3 substitution in the aromatic ring of the dipolarophile exhibited poor activity. The highly active compounds have 2-OH or 4-Cl substitution (polar groups) in their structure. Since these compounds have more polar substitution, it increases the hydrophilicity which increases their water solubility in the *in vitro* antibacterial activity assay. Bacterial cell membrane also was comprised of lipopolysaccharide which was hydrophilic in nature which further helps in the penetration of these polar compounds through this membrane. Other researchers and research performed by us also found that polar substitution in compounds enhances the anti-infective activity [16]. More over, hydrophilic/lipophilic balance (log P) of the compounds plays an important role in the absorption, distribution, metabolism and excretion of the compounds.

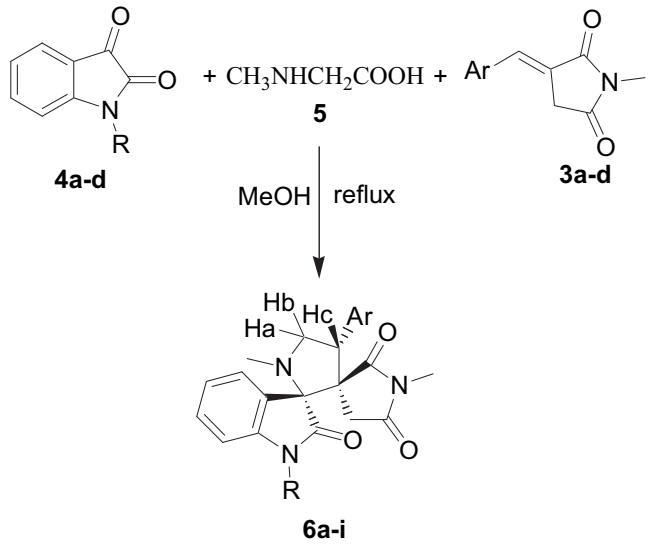
4. QSAR studies

In the case of *Salmonella typhi*, compounds **6b**, **6e**, **6g** and **6h** are most active and compounds **6c**, **6d** and **6f** are least active in this set.

Table 1
Synthesis of 3-benzylidene-1-methyl-pyrrolidine-2,5-dione **3**.

Entry	Ar	Product	Time (min)	Yield %[a]
1	2-OHC_6H_4	**3a**	30	78
2	4-$OMeC_6H_4$	**3b**	45	72
3	4-ClC_6H_4	**3c**	60	68
4	4-MeC_6H_4	**3d**	45	76

[a] Isolated yield after column chromatography.



Scheme 2. Synthesis of dispiropyrrolidines.

The QSAR equation developed for *S. typhi* and the statistics are given below:

$$Activity = 0.8185 - 0.0492 \times S\_ssO - 0.2135 \times Jurs - RPCS$$

$$r = 0.98, r^2 = 0.97, r^2_{adj} = 0.96, q^2 = 0.89,$$
$$F - \text{ratio} = 86.43, \text{error} = 0.03$$

The biparametric equation consists of two descriptors namely, S_ssO and Jurs-RPCS which are negatively correlated to activity.

Jurs-RPCS is a spatial descriptor and it is equal to the solvent accessible area of the most positive atom divided by Jurs-RPCG, where the later is calculated by considering the atomic partial charges. It is a measure of the relative positive surface charge of the molecule [17]. Outer membrane of the bacteria comprises of glycolipid and lipopolysacchride (LPS), which are hydrophilic in nature and it acts as a barrier for hydrophobic molecules. The inner leaflet comprises of mixture of phospholipids, which are hydrophobic in nature and they limit the penetration of hydrophilic compounds. Highly charged or polar low molecular weight compounds will have more permeability through the outer membrane than others [18]. Hence partial surface charge (Jurs-RPCS) appears in the QSAR. Our earlier research showed the contribution of other Jurs descriptors (Jurs-RPCG, Jurs-PPSA-3) towards the antibacterial activity against *S. typhi*. [19–21].

S_ssO is an electrotopological descriptor that describes the energy states of the O bonds, especially ether oxygen atoms in the molecule [22]. The compounds **6c**, **6d** and **6f** have ether linkage

Table 2
Synthesis of dispiropyrrolidines.

Entry	Dipolarophiles (**3**)	R	Product	Time (h)	Yield %[a]
1	**3a**	H	**6a**	3.0	75
2	**3a**	Me	**6b**	4.0	71
3	**3b**	H	**6c**	4.5	74
4	**3b**	Me	**6d**	3.0	72
5	**3a**	Propargyl	**6e**	6.0	64
6	**3b**	Bn	**6f**	3.5	76
7	**3c**	H	**6g**	4.0	66
8	**3c**	Me	**6h**	4.5	65
9	**3d**	H	**6i**	5.0	69

[a] Isolated yield after column chromatography.

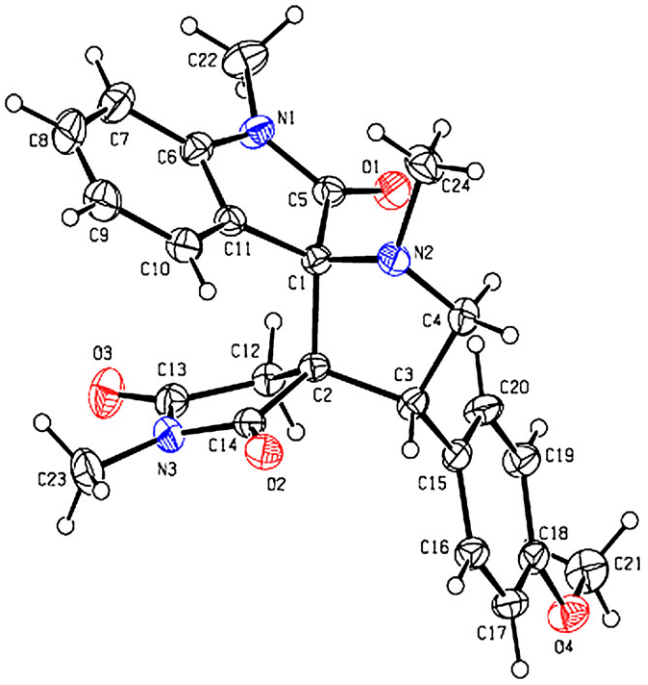


Fig. 1. ORTEP diagram of compound **6d**.

through their 4-OCH_3 substitution, hence they are least active. Table 4 lists the predicted and experimental activity (= −log (MIC)). Table 5 lists the numerical values of the descriptors used for developing the QSAR equation for *S. typhi*. Fig. 2 shows the plot relating the predicted activity with the experimental values for *S. typhi*.

In the case of *Proteus vulgaris*, compounds **6b**, **6e**, **6g** and **6h** are the most active and compounds **6c**, **6d** and **6f** are the least active. The QSAR equation developed for *P. vulgaris* and the corresponding statistics are given below:

$$Activity = 0.8148 - 0.0508 \times S\_ssO$$

$$r = 0.98, r^2 = 0.96,\ r^2_{adj} = 0.95, q^2 = 0.92,$$
$$F - \text{ratio} = 162.76, \text{error} = 0.03$$

The electrotopological descriptor, S_ssO is described before and once again it has a negative effect on activity. Compounds **6c**, **6d** and **6f** have ether linkage and have least activity. Their activities are highly correlated with S_ssO ($r = 0.99$). Table 6 lists the numerical values of the descriptors used for developing the QSAR equation for *P. vulgaris*. Fig. 3 shows the plot relating the predicted activity with the experimental values for *P. vulgaris*.

In the case of *Pseudomonas aeruginosa*, compounds **6a**, **6b**, **6e**, **6g** and **6h** are the most active and **6d**, **6f** and **6i** are the least active ones. The QSAR equation developed for *P. aeruginosa* and the corresponding statistics is given below:

$$Activity = 1.4142 - 1.5966 \times S\_sssCH - 0.0197 \times Shadow - YZ$$

$$r = 0.90, r^2 = 0.81, r^2_{adj} = 0.75, q^2 = 0.61,$$
$$F - ratio = 12.73, error = 0.11$$

Both the descriptors in this biparametric model have negative contribution towards activity. Shadow index is a molecular property describing its shape, conformation and orientation. Shadow-YZ denotes the area of the molecular shadow in the YZ plane [23]. Our earlier research also showed the negative contribution of shadow descriptors (shadow-Y length) towards the antibacterial activity of isoxazolidine derivatives against *P. aeruginosa* [24]. S_sssCH, is an electrotopological descriptor, that represents carbon atom which has three single bonds. This has a negative contribution to activity indicating that compounds with more S_sssCH will be less active. Table 7 lists the numerical values of the descriptors used for developing the QSAR equation for *P. aeruginosa*. Fig. 4 shows the plot relating the predicted activity with the experimental values for *P. aeruginosa*.

In the case of *Bacillus subtilis*, compounds **6e**, **6f** and **6h** are the most active and compounds **6a** and **6i** are the least active. The QSAR equation developed for antibacterial activity against *B. subtilis* and the statistics are given below:

$$Activity = -3.1291 + 0.5961 \times IC + 0.0164 \times shadow - YZ$$

$$r = 0.97, r^2 = 0.94,\ r^2_{\text{adj}} = 0.92, q^2 = 0.57,$$
$$F - \text{ratio} = 48.33, \text{error} = 0.03$$

IC belongs to the class of information − theoretic topological indices which are calculated by multiplying average information content with the total number of atoms. The average information content is obtained based on Shannon information theory and is calculated by the following formula [25,26].

$$^{k}I\overline{C} = -\sum \frac{n_i}{n} \log 2 \frac{n_i}{n}$$

Where n − total number of atoms in the molecule

n_i − number of atoms in i-th class

IC is also related to the lipophilicity of the molecule [19]. This descriptor shows a positive correlation with the activity. Increase in the IC value leads to increase in the antibacterial activity. The antibacterial activity against *B. subtilis* of the most active compounds and the IC values are found to be highly correlated ($r = 0.89$). Shadow-YZ contributes positively denoting that increase in area in YZ plane will lead to increase in activity. The antibacterial activity against *B. subtilis* of most active compounds and shadow-YZ are highly correlated ($r = 0.79$). This descriptor also appeared in the QSAR for *P. aeruginosa*. Table 8 lists the numerical values of the descriptors used for developing the QSAR equation for *B. subtilis*. Fig. 5 shows the plot relating the predicted activity with the experimental values for *B. subtilis*.

In the case of *Staphylococcus aureus*, compounds **6d**, **6e**, **6f** and **6h** are found to be the most active and compounds **6a**, **6b** and **6c** are the least active. The QSAR equation developed for the antibacterial activity against *S. aureus* and the statistics are given below:

Table 3
The observed antibacterial activities of dispiropyrrolidines.

Compounds	Antibacterial activity (MIC in μM)				
	B. subtilis	*S. aureus*	*P. aeruginosa*	*S. typhi*	*P. vulgaris*
6a	0.639	0.639	0.080	0.160	0.160
6b	0.617	0.617	0.077	0.154	0.154
6c	0.617	0.617	0.154	0.308	0.308
6d	0.596	0.298	0.298	0.298	0.298
6e	0.291	0.291	0.146	0.146	0.146
6f	0.504	0.252	0.252	0.252	0.252
6g	0.610	0.305	0.152	0.152	0.152
6h	0.590	0.295	0.147	0.147	0.147
6i	0.642	0.321	0.321	0.160	0.160
Ampicillin	0.168	0.084	0.168	0.084	0.168
Tetracycline	0.001	0.002	0.004	0.004	0.001

Table 4
Observed and predicted activities of dispiropyrrolidines.

Compounds	Antibacterial activity (−log (MIC))									
	B. subtilis		*S. aureus*		*P. aeruginosa*		*S. typhi*		*P. vulgaris*	
	Observed activity	Predicted activity	Observed activity	Predicted activity	Observed activity	Predicted activity	Observed activity	Predicted activity	Observed activity	Predicted activity
6a	0.1947	0.1950	0.1947	0.2001	1.0978	1.1209	0.7968	0.8007	0.7968	0.8148
6b	0.2100	0.1594	0.2100	0.2078	1.1131	1.0554	0.8121	0.8185	0.8121	0.8148
6c	0.2100	0.2081	0.2100	0.2886	0.8121	0.8145	0.5110	0.5588	0.5110	0.5467
6d	0.2248	0.2675	0.5258	0.4729	0.5258	0.5011	0.5258	0.5214	0.5258	0.5462
6e	0.5360	0.5173	0.5360	0.5366	0.8371	0.8832	0.8371	0.8185	0.8371	0.8148
6f	0.2972	0.3009	0.5982	0.5500	0.5982	0.6670	0.5982	0.5552	0.5982	0.5429
6g	0.2147	0.2082	0.5157	0.4703	0.8168	0.7631	0.8168	0.8185	0.8168	0.8148
6h	0.2293	0.2507	0.5304	0.5937	0.8314	0.6527	0.8314	0.8185	0.8314	0.8148
6i	0.1925	0.2108	0.4935	0.4121	0.4935	0.6663	0.7946	0.8137	0.7946	0.8148

$$Activity = -10.2792 + 1.6917 \times IC + 2.6087 \times CHI - V - 3\_C$$

$$r = 0.94, r^2 = 0.89,\ r^2_{adj} = 0.85, q^2 = 0.71,$$
$$F - \text{ratio} = 23.11, \text{error} = 0.06$$

Information content shows a positive correlation and it also appeared in the QSAR of *B. subtilis*. The antibacterial activity against *S. aureus* of the most active compounds showed positive correlation with IC ($r = 0.62$). A relationship between IC and the antibacterial activity against *S. aureus* is also observed by us earlier [19]. CHI-V-3_C belongs to the Kier and Hall molecular connectivity indices and the indices. It explains the branching, ring structures and flexibility of the molecule. CHI-V-3_C has a positive correlation with the activity. Table 4 shows the observed and predicted activities of dispiropyrrolidines against all the five organisms. Table 9 lists the numerical values of the descriptors used for developing the QSAR equation for *S. aureus*. Fig. 6 shows the plot relating the predicted activity with the experimental values for *S. aureus*.

5. Conclusion

In summary, we have reported the synthesis and characterisation of nine dispiropyrrolidines. These compounds were evaluated for their activities against five bacteria. Among these nine compounds tested, compounds **6e** and **6h** exhibited reasonably good activity and **6c** exhibited least activity. QSAR was developed by relating antibacterial activity of dispiropyrrolidines and their physicochemical descriptors. Statistical measures obtained for all five QSAR equations were found to be satisfactory. Electrotopological descriptors, Jurs descriptors, topology, electrostatic forces, shape and hydrophobic nature of the molecule were found to contribute to the antibacterial activities. All the above said parameters are related to the solubility and hydrophobic/hydrophilic nature of the compounds. The drug solubility affects its penetration through bacterial cell membrane. Bacterial cell membrane is a very complicated structure where the outer surface is hydrophilic in nature and inner leaflet is lipophilic in nature. Thus the former acts as a barrier for hydrophobic drugs and the later acts as barrier for hydrophilic drugs. An ideal candidate for antibacterial therapy should posses a good hydrophilic/lipophilic balance. This QSAR study showed the contribution of the above said descriptors.

Table 5
Lists the numerical values of the descriptors used for developing the QSAR equation for *S. typhi*.

Compounds	S_ssO	Jurs-RPCS
6a	0.0000	0.0833
6b	0.0000	0.0000
6c	5.2782	0.0000
6d	5.2882	0.1729
6e	0.0000	0.0000
6f	5.3522	0.0000
6g	0.0000	0.0000
6h	0.0000	0.0000
6i	0.0000	0.0226

6. Experimental

Melting points were determined in capillary tubes and are uncorrected. IR spectra were taken as neat for liquid compounds and as KBr pellets for solids on a Perkin Elmer Spectrum RXI FT-IR. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded in $CDCl_3$ and DMSO-d_6 solutions with TMS as an internal standard on a JEOL instrument. Mass spectra were recorded on a Thermo Finnigan LCQ Advantage MAX 6000 ESI spectrometer. Elemental analysis data were recorded using Thermo Finnigan FLASH EA 1112 CHN analyzer.

6.1. Experimental procedure for the synthesis of dispiropyrrolidines derivatives (*6a–i*)

A mixture of isatin **4a–d** (1 mmol), sarcosine **5** (1 mmol), and 3-benzylidene-1-methyl-pyrrolidine-2,5-dione **3a–d** (1 mmol) was refluxed in methanol (10 ml). Completion of the reaction was evidenced by TLC analysis. The solvent was removed under vacuo and the crude product was subjected to column chromatography using ethyl acetate: petroleum ether (2:8) as an eluent to afford pure dispiropyrrolidines **6a–i**.

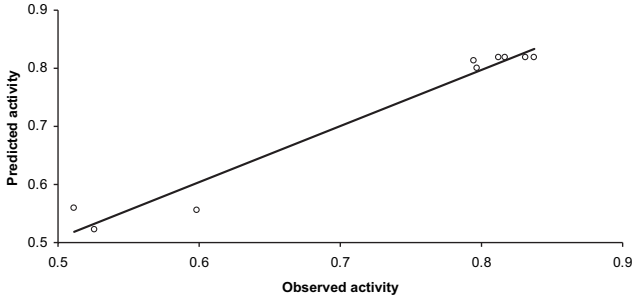


Fig. 2. Shows the plot relating the predicted activity with the experimental values for *S. typhi*.

Table 6
Lists the numerical values of the descriptors used for developing the QSAR equation for *P. vulgaris*.

Compounds	S_ssO
6a	0.0000
6b	0.0000
6c	5.2782
6d	5.2882
6e	0.0000
6f	5.3522
6g	0.0000
6h	0.0000
6i	0.0000

Table 7
Lists the numerical values of the descriptors used for developing the QSAR equation for *P. aeruginosa*.

Compounds	S_sssCH	Shadow-YZ
6a	−0.5213	57.1392
6b	−0.5198	60.3422
6c	−0.3271	56.9542
6d	−0.2105	63.4080
6e	−0.5714	73.2639
6f	−0.3625	67.3091
6g	−0.3305	59.8376
6h	−0.3290	65.3177
6i	−0.2718	59.9963

*6.1.1. 4′-(2-Hydroxyphenyl)-1′,1″-dimethyldispiro[indoline-3,2′-pyrrolidine-3′,3″-pyrrolidine]-2,2″,5″-trione (**6a**)*

Colourless solid; mp 236–238 °C; IR (cm^{-1}): 3412, 2995, 1782, 1705, 1602, 1441, 1157; ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.97 (d, 1H, J = 17.6 Hz, C*H*HCONMe), 2.02 (d, 1H, J = 17.6 Hz, CH*H*CONMe), 2.04 (s, 3H, NCH_3), 2.57 (s, 3H, NCH_3), 3.25 (t, 1H, J = 8.4 Hz, *H*a), 4.01 (t, 1H, J = 9.9 Hz, *H*b), 4.34 (t, 1H, J = 9.9 Hz, *H*c), 6.73–6.76 (m, 2H, Ar-*H*), 6.82 (t, 1H, J = 6.9 Hz, Ar-*H*), 6.93 (t, 1H, J = 7.6 Hz, Ar-*H*), 7.07 (t, 1H, J = 6.9 Hz, Ar-*H*), 7.17–7.21 (m, 2H, Ar-*H*), 7.42 (d, 1H, J = 7.6 Hz, Ar-*H*), 9.59 (s, 1H, OH), 10.56 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.9, 35.4, 36.0, 41.9, 54.9, 59.6, 77.9, 110.4, 114.9, 119.8, 122.5, 124.7, 125.0, 126.7, 128.6, 128.9, 130.6, 143.7, 156.1, 175.4, 177.4, 180.2; MS m/z = 392 M^+ + 1; Anal. Calcd for $C_{22}H_{21}N_3O_4$ (391.15): C, 67.51; H, 5.41; N, 10.74. Found: C, 67.46; H, 5.45; N, 10.61.

*6.1.2. 4′-(2-Hydroxyphenyl)-1,1′,1″-trimethyldispiro[indoline-3,2′-pyrrolidine-3′,3″-pyrrolidine]-2,2″,5″-trione (**6b**)*

Orange solid; mp 198–200 °C; IR (cm^{-1}): 3421, 2994, 1789, 1702, 1601, 1444, 1167; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.10 (brs, 1H), 2.13–2.17 (m, 4H, NCH_3, C*H*HCONMe), 2.33 (d, 1H, J = 19.1 Hz, CH*H*CONMe), 2.68 (s, 3H, NCH_3), 3.14 (s, 3H, NCH_3), 3.49 (t, 1H, J = 8.4 Hz, *H*a), 4.16 (t, 1H, J = 9.2 Hz, *H*b), 4.59 (t, 1H, J = 9.2 Hz, *H*c), 6.79 (d, 2H, J = 7.6 Hz, Ar-*H*), 6.92 (t, 1H, J = 7.6 Hz, Ar-*H*), 7.07 (t, 1H, J = 7.6 Hz, Ar-*H*), 7.12 (t, 1H, J = 7.6 Hz, Ar-*H*), 7.31 (t, 1H, J = 7.6 Hz, Ar-*H*), 7.43 (d, 1H, J = 7.6 Hz, Ar-*H*), 7.47 (d, 1H, J = 7.6 Hz, Ar-*H*); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.9, 25.9, 35.4, 35.9, 55.1, 60.1, 78.3, 108.7, 116.0, 120.9, 123.3, 123.7, 123.9, 126.7, 128.9, 130.2, 130.5, 144.5, 154.9, 176.0, 176.1, 180.7; MS m/z = 406 M^+ + 1; Anal. Calcd for $C_{23}H_{23}N_3O_4$ (405.17): C, 68.13; H, 5.72; N, 10.36. Found: C, 68.41; H, 5.71; N, 10.16.

*6.1.3. 4′-(4-Methoxyphenyl)-1′,1″-dimethyldispiro[indoline-3,2′-pyrrolidine-3′,3″-pyrrolidine]-2,2″,5″-trione (**6c**)*

Colourless solid; mp 232–234 °C; IR (cm^{-1}): 3405, 2975, 1762, 1709, 1608, 1445, 1159; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.01 (s, 3H, NCH_3), 2.07 (d, 1H, J = 18.4 Hz, C*H*HCONMe), 2.38 (d, 1H, J = 18.3 Hz, CH*H*CONMe), 2.48 (s, 3H, NCH_3), 3.36 (t, 1H, J = 8.4 Hz, *H*a), 3.69 (s, 3H, OCH_3), 3.71 (t, 1H, J = 9.2 Hz, *H*b), 4.16 (t, 1H, J = 9.2 Hz, *H*c), 6.76 (d, 1H, J = 7.6 Hz, Ar-*H*), 6.88 (d, 1H, J = 8.4 Hz, Ar-*H*), 6.95 (t, 1H, J = 7.6 Hz, Ar-*H*), 6.99 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.17–7.21 (m, 2H, Ar-*H*), 7.30 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.57 (d, 1H, J = 8.4 Hz, Ar-*H*), 10.65 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.9, 35.1, 36.6, 48.3, 55.5, 58.8, 61.5, 77.9, 110.5, 114.6, 115.0, 122.4, 125.5, 126.5, 131.5, 132.7, 143.2, 159.0, 174.5, 177.6, 179.4; MS m/z = 406 M^+ + 1; Anal. Calcd for $C_{23}H_{23}N_3O_4$ (405.17): C, 68.13; H, 5.72; N, 10.36. Found: C, 67.91; H, 5.69; N, 10.51.

*6.1.4. 4′-(4-Methoxyphenyl)-1,1′,1″-trimethyldispiro[indoline-3,2′-pyrrolidine-3′,3″-pyrrolidine]-2,2″,5″-trione (**6d**)*

Colourless solid; mp 174–176 °C; IR (cm^{-1}): 3405, 2967, 1788, 1710, 1622, 1451, 1151; ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.96 (s, 3H, NCH_3), 2.03 (d, 1H, J = 18.4 Hz, C*H*HCONMe), 2.28 (d, 1H, J = 18.4 Hz, CH*H*CONMe), 2.50 (s, 3H, NCH_3), 3.07 (s, 3H, NCH_3), 3.39 (t, 1H, J = 8.4 Hz, *H*a), 3.70 (s, 3H, OCH_3), 3.74 (t, 1H, J = 9.2 Hz, *H*b), 4.15 (t, 1H, J = 9.2 Hz, *H*c), 6.89 (d, 2H, J = 8.4 Hz, Ar-*H*), 6.98 (d, 1H, J = 7.6 Hz, Ar-*H*), 7.04 (t, 1H, J = 7.6 Hz, Ar-*H*), 7.26 (d, 1H, J = 6.9 Hz, Ar-*H*), 7.31–7.33 (m, 3H, Ar-*H*); ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.9, 26.2, 35.1, 36.4, 48.8, 55.6, 58.8, 61.3, 77.7, 109.5, 114.6, 123.1, 124.7, 126.3, 130.1, 130.6, 131.5, 144.7, 159.0, 174.4, 175.6, 179.5; MS m/z = 420 M^+ + 1; Anal. Calcd for $C_{24}H_{25}N_3O_4$ (419.18): C, 68.72; H, 6.01; N, 10.02. Found: C, 68.78; H, 6.06; N, 10.14.

*6.1.5. 4′-(2-Hydroxyphenyl)-1′,1″-dimethyl-1-propargldispiro [indoline-3,2′-pyrrolidine-3′,3″-pyrrolidine]-2,2″,5″-trione (**6e**)*

Yellow solid; mp 178–180 °C; IR (cm^{-1}): 3422, 2998, 1785, 1712, 1622, 1449, 1154; ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.92 (s, 2H, CH_2CONMe), 2.01 (s, 3H, NCH_3), 2.61 (s, 3H, NCH_3), 3.17 (t, 1H, J = 2.3 Hz, C≡C*H*), 3.31 (t, 1H, J = 8.4 Hz, *H*a), 4.02 (t, 1H, J = 9.2 Hz, *H*b), 4.36 (t, 1H, J = 9.2 Hz, *H*c), 4.45 (s, 2H, NCH_2), 6.76 (d, 1H, J = 7.6 Hz, Ar-*H*), 6.84 (t, 1H, J = 6.9 Hz, Ar-*H*), 7.05–7.07 (m, 3H, Ar-*H*), 7.30 (d, 1H, J = 6.9 Hz, Ar-*H*), 7.35 (t, 1H, J = 8.4 Hz, Ar-*H*), 7.47 (d, 1H, J = 7.6 Hz, Ar-*H*), 9.62 (s, 1H, OH); ^{13}C NMR (125 MHz, DMSO-d_6):

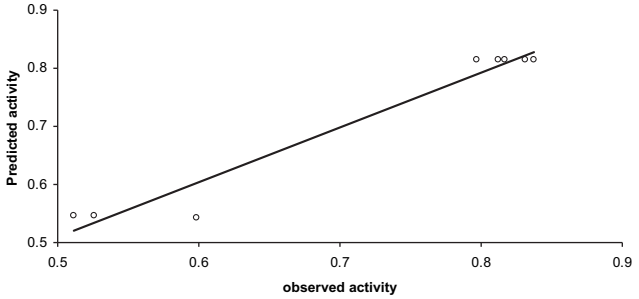


Fig. 3. Shows the plot relating the predicted activity with the experimental values for *P. vulgaris*.

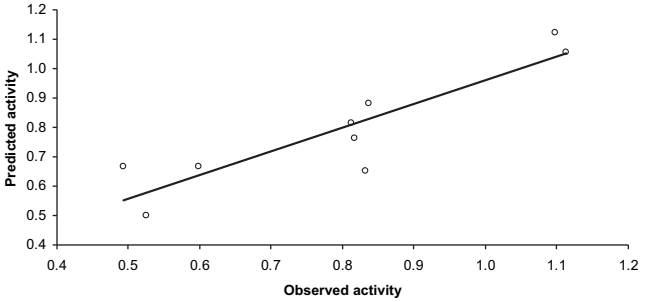


Fig. 4. Shows the plot relating the predicted activity with the experimental values for *P. aeruginosa*.

Table 8
Lists the numerical values of the descriptors used for developing the QSAR equation for *B. subtilis*.

Compounds	IC	Shadow-YZ
6a	4.0044	57.1392
6b	3.8566	60.3422
6c	4.0314	56.9542
6d	3.9536	63.4080
6e	4.1014	73.2639
6f	3.9023	67.3091
6g	3.9523	59.8376
6h	3.8729	65.3177
6i	3.9523	59.9963

Table 9
Lists the numerical values of the descriptors used for developing the QSAR equation for *S. aureus*.

Compounds	IC	CHI-V-3_C
6a	4.0044	1.4203
6b	3.8566	1.5191
6c	4.0314	1.4367
6d	3.9536	1.5578
6e	4.1014	1.4864
6f	3.9023	1.6206
6g	3.9523	1.5576
6h	3.8729	1.6564
6i	3.9523	1.5353

δ 24.9, 28.8, 35.2, 35.4, 42.3, 54.9, 59.7, 74.6, 77.5, 78.4, 110.1, 114.9, 119.8, 123.6, 124.2, 124.5, 126.7, 128.7, 128.8, 130.7, 143.2, 156.2, 175.0, 175.2, 180.1; MS m/z = 430 M^+ + 1; Anal. Calcd for $C_{25}H_{23}N_3O_4$ (429.17): C, 69.92; H, 5.40; N, 9.78. Found: C, 69.74; H, 5.42; N, 9.84.

*6.1.6. 1-Benzyl-4′-(4-Methoxyphenyl)-1′,1″-dimethyldispiro [indoline-3,2′-pyrrolidine-3′,3″-pyrrolidine]-2,2″,5″-trione (**6f**)*

Yellow solid; mp 104–106 °C; IR (cm^{-1}): 3412, 2998, 1786, 1705, 1602, 1441, 1158; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.01 (s, 3H, NCH_3), 2.10 (d, 1H, J = 18.3 Hz, C*H*HCONMe), 2.42 (d, 1H, J = 18.4 Hz, CH*H*CONMe), 2.49 (s, 3H, NCH_3), 3.43 (t, 1H, J = 8.4 Hz, *H*a), 3.69 (s, 3H, OCH_3), 3.79 (t, 1H, J = 9.2 Hz, *H*b), 4.24 (t, 1H, J = 9.2 Hz, *H*c), 4.88 (s, 2H, NCH_2Ph), 6.88–6.92 (m, 3H, Ar-*H*), 6.99–7.03 (m, 1H, Ar-*H*), 7.22–7.33 (m, 9H, Ar-*H*); ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.9, 35.2, 36.4, 43.4, 48.4, 55.5, 58.7, 61.6, 77.6, 110.1, 114.6, 123.2, 124.8, 126.6, 128.0, 129.2, 129.9, 130.6, 131.5, 132.7, 136.7, 143.7, 159.1, 174.3, 175.7, 179.2; MS m/z = 496 M^+ + 1; Anal. Calcd for $C_{30}H_{29}N_3O_4$ (495.22): C, 72.71; H, 5.90; N, 8.48. Found: C, 72.67; H, 5.91; N, 8.64.

*6.1.7. 4′-(4-Chlorophenyl)-1′,1″-dimethyldispiro[indoline-3,2′-pyrrolidine-3′,3″-pyrrolidine]-2,2″,5″-trione (**6g**)*

Colourless solid; mp 220–222 °C; IR (cm^{-1}): 3412, 2985, 1782, 1710, 1608, 1445, 1147; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.01 (s, 3H, NCH_3), 2.05 (d, 1H, J = 18.4 Hz, C*H*HCONMe), 2.36 (d, 1H, J = 17.6 Hz, CH*H*CONMe), 2.46 (s, 3H, NCH_3), 3.42 (t, 1H, J = 8.4 Hz, *H*a), 3.70 (t, 1H, J = 9.2 Hz, *H*b), 4.22 (t, 1H, J = 8.4 Hz, *H*c), 6.78 (d, 1H, J = 7.6 Hz, Ar-*H*), 6.95 (t, 1H, J = 6.9 Hz, Ar-*H*), 7.14 (d, 1H, J = 6.9 Hz, Ar-*H*), 7.22 (t, 1H, J = 7.6 Hz, Ar-*H*), 7.39–7.43 (m, 4H, Ar-*H*), 10.69 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.9, 35.1, 36.8, 47.9, 59.1, 61.4, 78.1, 110.6, 122.5, 125.3, 126.3, 129.1, 130.6, 132.5, 132.6, 137.8, 143.2, 174.3, 177.6, 179.2; MS m/z = 410 M^+ + 1, 412 M^+ + 3; Anal. Calcd for $C_{22}H_{20}ClN_3O_3$ (409.12): C, 64.47; H, 4.92; N, 10.25. Found: C, 64.61; H, 4.97; N, 10.17.

*6.1.8. 4′-(4-Chlorophenyl)-1,1′,1″-trimethyldispiro[indoline-3,2′-pyrrolidine-3′,3″-pyrrolidine]-2,2″,5″-trione (**6h**)*

Yellow solid; mp 226–228 °C; IR (cm^{-1}): 3408, 2996, 1782, 1717, 1609, 1445, 1157; ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.96 (s, 3H, NCH_3), 2.02 (d, 1H, J = 18.4 Hz, C*H*HCONMe), 2.27 (d, 1H, J = 18.3 Hz, CH*H*CONMe), 2.48 (s, 3H, NCH_3), 3.09 (s, 3H, NCH_3), 3.45 (t, 1H, J = 8.4 Hz, *H*a), 3.72 (t, 1H, J = 9.2 Hz, *H*b), 4.20 (t, 1H, J = 9.2 Hz, *H*c), 7.00 (d, 1H, J = 7.6 Hz, Ar-*H*), 7.04 (t, 1H, J = 7.6 Hz, Ar-*H*), 7.22 (d, 1H, J = 7.6 Hz, Ar-*H*), 7.33 (t, 1H, J = 7.6 Hz, Ar-*H*), 7.40 (d, 2H, J = 8.4 Hz, Ar-*H*), 7.46 (d, 2H, J = 8.4 Hz, Ar-*H*); ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.9, 26.2, 35.1, 36.7, 48.4, 59.0, 61.3, 77.8, 109.8, 123.2, 124.5, 126.1, 129.1, 130.8, 132.5, 132.7, 137.6, 143.4, 174.2, 175.6, 179.2; MS m/z = 424 M^+ + 1, 426 M^+ + 3; Anal. Calcd for $C_{23}H_{22}ClN_3O_3$ (423.13): C, 65.17; H, 5.23; N, 9.91. Found: C, 65.27; H, 5.16; N, 9.98.

*6.1.9. 4′-(4-Methylphenyl)-1′,1″-dimethyldispiro[indoline-3,2′-pyrrolidine-3′,3″-pyrrolidine]-2,2″,5″-trione (**6i**)*

Yellow solid; mp 216–218 °C; IR (cm^{-1}): 3402, 2998, 1782, 1710, 1602, 1439, 1157; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.01 (s, 3H, NCH_3), 2.05 (d, 1H, J = 18.4 Hz, C*H*HCONMe), 2.24 (s, 3H, NCH_3), 2.41 (d, 1H, J = 18.3 Hz, CH*H*CONMe), 2.47 (s, 3H, NCH_3), 3.35 (t, 1H, J = 8.4 Hz, *H*a), 3.74 (t, 1H, J = 9.2 Hz, *H*b), 4.18 (t, 1H, J = 8.4 Hz, *H*c), 6.77 (d, 1H, J = 7.6 Hz, Ar-*H*), 6.95 (t, 1H, J = 7.6 Hz, Ar-*H*), 7.12–7.25 (m, 6H, Ar-*H*), 10.64 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 21.2, 24.9, 35.1, 36.8, 48.6, 58.6, 61.5, 77.9, 110.5, 122.4, 125.5, 126.6, 129.8, 130.3, 130.5, 135.4, 137.1, 143.2, 174.4, 177.5, 179.4; MS m/z = 390 M^+ + 1; Anal. Calcd for $C_{23}H_{23}N_3O_3$ (389.17): C, 70.93; H, 5.95; N, 10.79. Found: C, 71.09; H, 5.94; N, 10.61.

6.2. Materials and methods for the antibacterial activity

B. subtilis NCIM 2718, *S. aureus* NCIM5021, *S. typhi* NCIM2501, *P. aeruginosa* NCIM 5029 and *P. vulgaris* NCIM2813 were purchased from National Chemical Laboratory, Pune, India and the reference antibiotics were purchased from Hi-media labs (Mumbai, India).

6.2.1. Antibacterial evaluation

Minimum Inhibitory Concentration (MIC) was determined by microdilution broth assay method [27] as reported by Sarker et al.

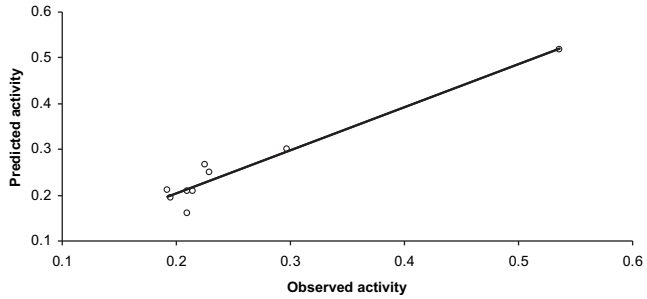


Fig. 5. Shows the plot relating the predicted activity with the experimental values for *B. subtilis*.

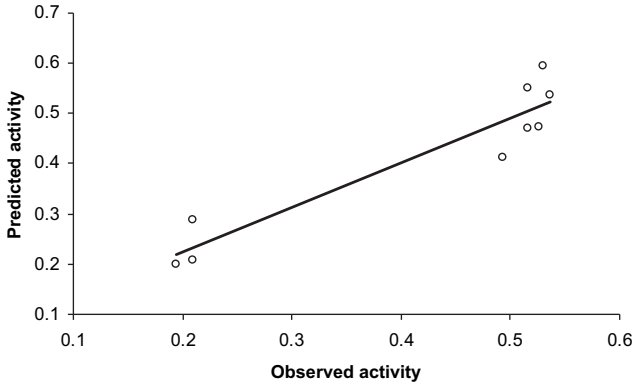


Fig. 6. Shows the plot relating the predicted activity with the experimental values for *S. aureus*.

[28] with slight modifications, using resazurin dye as an indicator. Bacterial strains were cultured in Muller hinton broth to reach a final inoculum size of 5×10^5 CFU/ml 10 mg of dispiropyrrolidines were dissolved in absolute ethanol to reach a final volume of 1 ml [29]. This solution is serially diluted and added to successive wells in a 96 well microtiter plate and incubated at 37 °C with respective micro-organisms for a period of 18 h. Growth and sterility controls were maintained for all the experiment. Test compounds serially diluted with ethanol were used (uninoculated dilution) to determine if they precipitated out during the course of the experiments. After 18 h, 10 μL of 0.01% resazurin solution was added to the wells and incubated for 2 h. A blank assay with ethanol was carried out. The color change was assessed visually. Blue color showed that the compound inhibited the microorganism and a change in color from blue to pink showed the growth of the organism.

6.2.2. Modelling studies

The chemical structures of nine of the dispiropyrrolidines were sketched and with the help of Cerius2 software (Accelrys, U.S.A), their energies were minimized using consistent valence force field (CVFF). This force field was parameterized using peptide and protein structures and is suitable for small molecules [30]. To facilitate the QSAR studies the antibacterial activity (minimum inhibitory concentration in μM) is converted as −log (MIC) concentration.

Two hundred and forty-nine physicochemical properties (descriptors), which include electronic, quantum mechanical, topological, spatial, structural, thermodynamic properties for these nine structures, were determined. Several references deal with a detailed description of these descriptors [31,32]. The Cerius2 software is capable of developing the best QSAR using GFA (Genetic Function Approximation) method. It can short list the descriptors from this large pool. The quality of the regression models were tested using various statistical parameters such as r^2, r^2_{adj}, cross-validated r^2 (q^2), *F*-ratio, predicted residual sum of squares (PRESS) and standard error of estimate. r^2 measures the strength of the linear relationship between the independent and the dependent variables. It is calculated as $r^2 = 1 - \mathrm{SSE/TSS}$, where SSE (sum of squares of the error) $= \Sigma (Y_{data} - Y_{model})^2$. TSS(total sum of squares) $= \sum (Y_{data} - \overline{Y})^2$. $\overline{Y}$ is the average of all the Y data. Y_{data} and Y_{model} are the observed and model predicted activity values, respectively. Adjusted r^2 is a modification to r^2 that adjusts for the number of independent terms in a model. The adjusted r^2 will always be less than or equal to r^2. It is calculated as $r^2_{adj} = 1 - (n - 1/n - p)(1 - r^2)$ where n is total number of observations and p is total number of terms in the model (including the constant). Cross-validated $r^2(q^2) = 1 -$ PRESS/TSS. Predicted sum of squares (PRESS) is the sum of the squared differences between the actual and predicted values for the dependent variable using regression equation developed by leaving one data point at a time. This LOO (leave one out) method is called internal validation technique to determine the predictive capability of the model. *F*-test is meant to find the significance of the regression model and it is performed by comparing the ratio of the variances between regression and the error (*F*-value) with the F-table value for $n - p - 1$ degrees of freedom. An F-value greater than the *F*-table value indicates that the regression relations is a not a chance fit but is a statistically significant occurrence [33].

Acknowledgements

One of the authors K. Karthikeyan thanks the Council of Scientific and Industrial Research, New Delhi, India for the research fellowship.

References

[1] (a) R. Huisgen, in: A. Padwa (Ed.), 1,3-Dipolar Cycloaddition Chemistry, vols. 1 and 2, Wiley, New York, 1984;
(b) O. Tsuge, S. Kanemasa, in: A.R. Katritzky (Ed.), Advances in Heterocyclic Chemistry, vol. 45, Academic Press, San Diego, 1989, pp. 231–252;
(c) R. Grigg, V. Sridharan, in: D.P. Curran (Ed.), Advances in Cycloaddition, vol. 3, Jai Press, London, 1993, pp. 161–180.
[2] (a) J.R. Liddell, Nat. Prod. Rep. 13 (1996) 187–193;
(b) J.R. Liddell, Nat. Prod. Rep. 15 (1998) 363–370.
[3] (a) T. Hartmann, L. Witte, in: S.W. Pelletier (Ed.), Chemistry, Biology and Chemoecology of Pyrrolizidine Alkaloids, Alkaloids: Chemical and Biological Perspectives, vol. 9, Pergamon Press, 1995;
(b) R.J. Molineux, in: S.W. Pelletier (Ed.), Alkaloids: Chemical and Biological Perspective, Wiley, New York, 1987 (Chapter 1);
(c) S. Fujimori, Jpn. Patent Appl. 88-2912. Chem. Abstr. 112 (1990) 98409.
[4] (a) R.R. Kumar, S. Perumal, P. Senthilkumar, P. Yogeeswari, D. Sriram, Tetrahedron 64 (2008) 2962–2971;
(b) R.R. Kumar, S. Perumal, P. Senthilkumar, P. Yogeeswari, D. Sriram, Eur. J. Med. Chem. 44 (2009) 3821–3829;
(c) R.R. Kumar, S.M. Rajesh, S. Perumal, D. Banerjee, P. Yogeeswari, D. Sriram, Eur. J. Med. Chem. 45 (2010) 411–422.
[5] (a) A. Dell, D.H. Williams, H.R. Morris, G.A. Smith, J. Feeney, G.C.K. Roberts, J. Am. Chem. Soc. 97 (1975) 2497–2502;
(b) S. Sapo, O. Shiratori, K. Katagiri, J. Antibiot. 20 (1967) 270;
(c) C. Bailly, S. Echepare, F. Gago, M. Waring, Anti-Cancer Drug Des 14 (1999) 291–303.
[6] A.P. Kozikowski, Acc. Chem. Res. 17 (1984) 410–416.
[7] R.K. Howe, B.R. Shelton, J. Org. Chem. 55 (1990) 4603–4607.
[8] M. De Amici, C. De Michelli, V. Misani, Tetrahedron 46 (1990) 1975–1986.
[9] V.L. Cohen, E.E. Kleinmann, PCT. Int. Appl.. W.O. 24192. Chem. Abstr 123 (1995) 296610t.
[10] (a) W.A. Carroll, P.A. Grieco, J. Am. Chem. Soc. 115 (1993) 1164–1165;
(b) W.G. Earley, T. Oh, L.E. Overman, Tetrahedron Lett. 29 (1988) 3785–3788;
(c) Y. Ban, N. Taga, T. Oishi, Chem. Pharm. Bull. 24 (1976) 736–751;
(d) Y. Ban, M. Seto, T. Oishi, Chem. Pharm. Bull. 23 (1975) 2605–2613.
[11] (a) Y. Ban, N. Taga, T. Oishi, Tetrahedron Lett. 15 (1974) 187–190;
(b) E.E. Van Tamelen, J.P. Yardley, M. Miyano, W.B. Hinshaw Jr., J. Am. Chem. Soc. 91 (1969) 7333–7341.
[12] H. Kabat, E.F. Stohlman, M.I. Smith, J. Pharmacol. 80 (1994) 160–170.
[13] W.G. Rajeswaran, R.B. Labroo, L.A. Cohen, J. Org. Chem. 64 (1999) 1369–1371.
[14] (a) K. Karthikeyan, P.T. Perumal, S. Etti, G. Shanmugam, Tetrahedron 63 (2007) 10581–10586;
(b) K. Karthikeyan, T.V. Seelan, K.G. Lalitha, P.T. Perumal, Bioorg. Med. Chem. Lett. 19 (2009) 3370–3373;
(c) K. Karthikeyan, R.S. Kumar, D. Muraidharan, P.T. Perumal, Tetrahedron Lett. 50 (2009) 7175–7179;
(d) M. Damodiran, D. Muraidharan, P.T. Perumal, Bioorg. Med. Chem. Lett. 19 (2009) 3611–3614.
[15] S. Nirmala, K. Karthikeyan, E.T.S. Kamala, L. Sudha, P.T. Perumal, Acta Crystallogr. E65 (2009) o1655.
[16] (a) Z. Zhong, R. Xing, S. Liu, L. Wang, S. Cai, P. Li, Carbohydr. Res. 343 (2008) 566–570;
(b) P.M. Sivakumar, S.P. Seenivasan, V. Kumar, M. Doble, Bioorg. Med. Chem. Lett. (2010).doi:10.1016/j.bmcl.2010.03.083;
(c) P.M. Sivakumar, S.P. Seenivasan, V. Kumar, M. Doble, Bioorg. Med. Chem. Lett. 17 (2010) 1695–1700.
[17] D.T. Stanton, P.C. Jurs, Anal. Chem. 62 (1990) 2323–2329.
[18] A. Wiese, M. Munstermann, T. Gutsmann, B. Lindner, K. Kawahara, U. Zahringer, U. Seydel, J. Membr. Biol. 162 (1998) 127–138.
[19] P.M. Sivakumar, G. Sheshayan, M. Doble, Chem. Biol. Drug Des. 71 (2008) 303–313.
[20] P.M. Sivakumar, S.K.G. Babu, M. Doble, Chem. Biol. Drug Des. 71 (2008) 447–463.
[21] P.M. Sivakumar, S. Priya, M. Doble, Chem. Biol. Drug Des. 73 (2009) 403–415.
[22] J.B. Bhonsle, A.K. Bhattacharjee, R.K. Gupta, J. Mol. Model. 13 (2007) 179–208.
[23] R.H. Rohrbaugh, P.C. Jurs, Anal. Chim. Acta 199 (1987) 99–109.
[24] M. Damodiran, P.M. Sivakumar, R. Senthil Kumar, D. Muralidharan, B.V.N. Phani Kumar, M. Doble, P.T. Perumal, Chem. Biol. Drug Des. 74 (2009) 494–506.
[25] L.B. Kier, J. Pharm. Sci. 69 (1980) 807–810.
[26] D. Bonchev, Information Theoretic Indices for Characterization of Chemical Structure. Wiley-Interscience, New York, 1983.
[27] F. Candan, M. Unlu, B. Tepe, D. Daferera, M. Polissiou, A. Sokmen, H.A. Akpulat, J. Ethnopharmacol. 87 (2003) 215–220.
[28] S.D. Sarker, L. Nahar, Y. Kumarasamy, Methods 42 (2007) 321–324.
[29] E.S. Charles, V.K. Agrawal, S. Sharma, R.N. Iyer, O.P. Srivastava, Eur. J. Med. Chem. 14 (1979) 435–438.
[30] W.D. Cornell, P. Cieplak, C.I. Bayly, I.R. Gould, K.M. Merz, D.M. Ferguson, D. C. Spellmeyer, T. Fox, J.W. Caldwell, P.A. Kollman, J. Am. Chem. Soc. 117 (1995) 5179–5197.
[31] (a) R. Todeschini, M. Lasagni, E. Marengo, J. Chemom. 8 (1994) 263–272;
(b) R. Todeschini, V. Consonni, Handbook of Molecular Descriptors. Wiley-VCH, Weinheim, 2000.
[32] M. Karelson, Molecular Descriptors in QSAR/QSPR. Wiley Interscience, New York, 2000.
[33] A.R. Leach, Molecular Modelling Principles and Applications, second ed.. Pearson Education EMA, U.K, 2001, 698–702.