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Expeditious synthesis, antibacterial activity evaluation and GQSAR studies of 3-bisoxindoles, 2-oxindolyl-2-hydroxyindan-1,3-diones and 2-oxindolyl-2-hydroxyacenaphthylen-1-ones†

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Neelakandan Vidhya Lakshmi,[a] Ponnurengam Malliappan Sivakumar,[b] Doraiswamy Muralidharan,[a] Mukesh Doble[b] and Paramasivan T. Perumal*[a]

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The rhodium(II)-catalyzed one-pot three-component reaction of 3-diazooxindole, benzyl alcohol and isatin or ninhydrin or acenaphthenequinone was explored to synthesize 3-bisoxindoles, 2-oxindolyl-2-hydroxyindan-1,3-diones and 2-oxindolyl-2-hydroxyacenaphthylen-1-ones with vicinal stereo centres, respectively through oxonium ylides. Their antibacterial activities against *Staphylococcus aureus* NCIM 5021, *Escherichia coli* NCIM 2931, *Pseudomonas aeruginosa* NCIM 5029, *Salmonella typhimurium* NCIM 2501 and *Proteus vulgaris* NCIM 2813 were evaluated by a microdilution method. Group-based quantitative structure activity relationships (GQSAR) for all antibacterial activities were developed. The statistical measures namely r^2 (0.66–0.85), r^2 adj (0.63–0.84), q^2 (0.54–0.79) and F-ratio (11.63–34.17) were found to be satisfactory. All of the test and training set compounds were found to be within a 99% confidence level. Descriptors pertaining to hydrophobicity, topology and electrostatic interactions of the substitutions were found to contribute to their activities.

Introduction

Multicomponent reactions (MCRs) are flexible, selective, convergent and atom-efficient processes that facilitate the construction of complex molecules in a single step.[1] Oxindoles incorporated with a quaternary stereogenic centre at C3 are attractive targets in organic synthesis since they display a fascinating array of biological applications.[2] Oxindoles have proven anti-infective properties. Strigacova *et al.* tested the antifungal activity of these compounds against filamentous fungi like *R. oryzae*, *B. cinerea*, *F. moniliforme*, *A. flavus*, *T. interdigitale*, *M. gypseurn*.[2c] Chande *et al.* reported the antitubercular activity of spirooxindoles against *M. tuberculosis* H_{37}Rv strain.[2d] They also possess antibacterial activity, which was explored by several researchers.[2e–h] Moreover oxindoles exhibit a plethora of activities, such as anticancer, anti-protozoal, anti-inflammatory, spermicidal activities, and also act against progesterone receptors.[2i–2l] Among the oxindole derivatives, the 3-substituted 3-hydroxyindolin-2-one nucleus represents an important structural motif present in an extensive number of biologically active alkaloids, such as TMC-95s,[3] Welwitindolinone C,[4] Celogentin K,[5] Convolutamydines[6] and SM-130686.[7] There are many methods known to synthesize 3-substituted 3-hydroxyindolin-2-ones *via* nucleophilic addition to isatins, which generate only one stereocentre.[8]

Many studies of transition metal-catalyzed decomposition of diazocarbonyl compounds have focused on cyclopropanation, X–H (X = C, N, O, Si, S) insertion and ylide generation,[9–11] and these reactions have also been used to prepare several alkaloids, such as Aspidosperma, Kopsifoline and Aspidophytine.[12] The chemistry of oxonium ylides is an area of continuing interest due to its wide synthetic utility.[13–15] Several methods were known for the *in situ* generation of oxonium ylides from rhodium carbenoids and for their synthetic uses.[14–16] To the best of our knowledge, this is the first time 3-diazooxindole has been used for the one-pot multicomponent synthesis of 3-bisoxindoles, which contain a 3-hydroxyindoline-2-one moiety with contiguous stereo carbon centres with rhodium(II) acetate as a catalyst *via in situ* generation and trapping of oxonium ylides. We herein disclose a method for the generation of oxonium ylides from 3-diazooxindole and benzyl alcohols *via* rhodium carbenoids, which undergo nucleophilic addition to isatin, ninhydrin and acenaphthenequinone to synthesize 3-bisoxindoles, which contain a 3-hydroxyindoline-2-one moiety, 2-oxindolyl-2-hydroxyindan-1,3-dione derivatives and 2-oxindolyl-2-hydroxyace-

[a]Organic Chemistry Division, Central Leather Research Institute (CSIR), Adyar, Chennai-600 020, India. E-mail: ptperumal@gmail.com; Tel: +91 44 24913289
[b]Department of Biotechnology, Indian Institute of Technology Madras, Adyar, Chennai-600036, India

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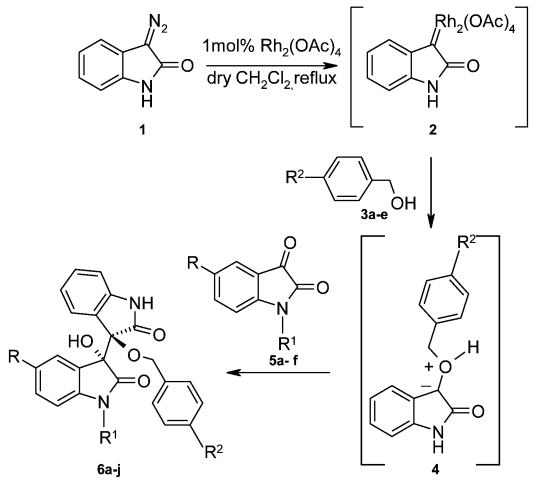


Scheme 1 Synthesis of 3-bisoxindoles (**6a–j**).

naphthylen-1-ones with vicinal stereocentres, respectively. Their antibacterial activity against five of the Gram +ve, namely *Staphylococcus aureus* and Gram –ve bacterial strains, namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Proteus vulgaris* were studied.

Results and discussion

We started our initial study with rhodium(II) acetate (1 mol%) catalyzed three component reaction of 3-diazooxindole **1**,[17] benzyl alcohol **3a** and isatin **5a** in dichloromethane, which afforded highly functionalized bisoxindole **6a** with adjacent stereocentres in just a single step. Optimum yields were obtained by slow addition of 3-diazooxindole **1** to the refluxing solution of isatin **5a**, benzyl alcohol **3a** and rhodium(II) acetate in dry dichloromethane under argon atmosphere. The solid precipitated from the reaction was filtered and recrystallized from ethanol to furnish the compound **6a** as a single product. The synthetic protocol is remarkably simple and requires no further purification processes like column chromatography (Scheme 1).

Encouraged by the results obtained in the above reaction, we were further interested to carry out the reactions of 3-diazooxindole **1** with various substituted benzyl alcohols **3a–e** and isatin derivatives **5a–f**. All of the reactions proceeded smoothly in 90–100 min to give the respective 3-bisoxindoles **6a–j** in good yields (82–90%). The reactions were diastereoselective, as only one isomer was obtained in all of the reactions (Table 1).

Based on the above results, a tentative mechanistic interpretation to explain the formation of the product **6** is proposed[13,18,19] (Scheme 2). The reaction can be considered to proceed *via* the initial formation of rhodium carbenoid **2**, which reacts with benzyl alcohol **3** to generate oxonium ylide **4**. The oxonium ylide **4** generated is trapped by an electrophile, such as isatin **5**, to afford the product **6**.

The O–H insertion side product **2a** from **1** and **3** was not formed due to the high electrophilicity of isatin **5**.[18,19] The high electrophilicity of the carbonyl group of isatin **5** accounts for the nucleophilic addition of oxonium ylide **4** to isatin **5**.

The structures of products **6a–j** were confirmed by spectral studies and elemental analysis. The ^{1}H NMR spectrum of compound **6b** exhibited characteristic singlets at δ 6.16, 10.16 and 10.44 ppm for hydroxy proton and –N*H* protons (D_2O exchangeable) of two oxindole rings, respectively. The peaks at δ 10.16 and 10.44 ppm confirm the incorporation of two oxindole rings in the structure. The ^{13}C NMR spectral analysis of compound **6b** showed peaks at δ 173.9 and 175.1 ppm for the presence of amide carbonyl groups of two oxindole rings. The mass spectrum of the compound displayed the molecular ion peak at *m*/*z* 416.87. The stereochemistry of the products **6a–j** were established through single-crystal X-ray analysis of the compound **6a**, which clearly illustrated the stereochemistry in Fig. 1.[20]

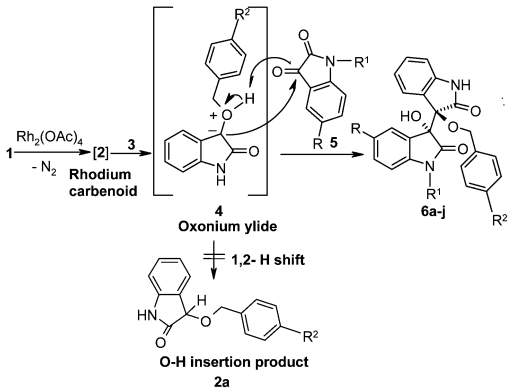
The promising results with isatin derivatives prompted us to explore further with ninhydrin **7** and acenaphthenequinone **9** under the same optimized conditions to provide 2-oxindolyl-2-hydroxyindan-1,3-diones **8a–e** and 2-oxindolyl-2- hydroxyacenaphthylen-1-ones **10a–e**, respectively (Scheme 3 and Scheme 4). The results are summarized in Table 2 and Table 3.

The ^{1}H NMR spectrum of **8d** exhibited a broad singlet at δ 6.51 due to the –OH proton and signal at δ 10.56 ppm for –N*H* proton (D_2O exchangeable).

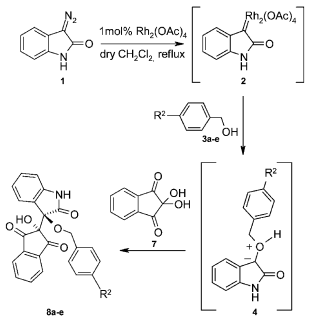
Table 1 Synthesis of 3-bisoxindole deivatives **6a–j**

Entry	Isatin (5)	R	R^1	Alcohol (3)	R^2	Product (6)[a]	Time (min)	Yield (%)[b]
1	**5a**	H	H	**3a**	H	**6a**	90	87
2	**5a**	H	H	**3b**	OMe	**6b**	90	90
3	**5a**	H	H	**3c**	Me	**6c**	90	89
4	**5a**	H	H	**3d**	Cl	**6d**	100	87
5	**5a**	H	H	**3e**	Br	**6e**	90	83
6	**5b**	Cl	H	**3a**	H	**6f**	90	83
7	**5c**	Br	H	**3a**	H	**6g**	90	82
8	**5d**	I	H	**3a**	H	**6h**	90	82
9	**5e**	H	allyl	**3a**	H	**6i**	90	87
10	**5f**	H	methyl	**3a**	H	**6j**	90	86

[a] The products were characterized by IR, NMR, mass and elemental analysis. [b] Isolated yield after recrystallization.



Scheme 2 A plausible mechanism for the synthesis of 3-bisoxindoles **6a–j**.



Scheme 3 Synthesis of 2-oxindolyl-2-hydroxy-indan-1,3-diones **8a–e**.

Resonances at δ 173.2, 196.9 and 197.9 ppm (three carbonyl groups) were observed in ^{13}C NMR spectrum. The mass spectrum of **8d** displayed the molecular ion ($M^+ + H^+$) peak at m/z 434.6.

Biological evaluation

Various oxindoles synthesized with different substitutions in the R^1 and R^2 position, and their antibacterial activities in micromolar concentrations against five of the organisms by microdilution broth assay method[21] are shown in Table 4. In general compounds **6b**, **6d** and **6e** are found to be more active, while **6a**, **6c** and **10e** are found to be less active than other compounds. The most active compounds have an oxindole group in their R^1 position. Introduction of substitutions in the oxindole ring (compounds **6f–6j**) leads to a lowering of activity. The replacement of an oxindole derivative with indan-1,3-dione derivative (**8a–8e**) and acenaphthylen-1-ones (**10a–10e**) leads to a reduction in the antibacterial activity. The least active compounds, **6a** and **6c**, also have an oxindole group at their R^1 position, but the substitution at R^2 seems to determine the activity within 3-bisoxindoles. The highly active compounds, **6b**, **6d**, **6e**, have –OMe, –Cl and –Br substitution (polar groups) in their structure, which belong to the electron withdrawing substituents. Earlier researchers also found that the electron withdrawing substitutions present in the indoline derivatives showed good antibacterial and antifungal activities.[21] The electron withdrawing substituents reduce the lipophilicity of the compound and make them soluble in water. Thus, the bioavailability and antibacterial activity of the compounds are increased. A bacterial cell membrane is also comprised of lipopolysaccharide, which is hydrophilic in nature, which further helps the penetration of these polar compounds through this membrane. Other researchers and research performed by us also found that polar substitutions in compounds enhance the anti-infective activity.[22–24] Moreover, hydrophilic/lipophilic balance (log P) of the compounds plays an important role in the absorption, distribution, metabolism and excretion of the compounds.

GQSAR studies

GQSAR is an ingenius method, which relates the biological response variation to the molecular fragments of interest. GQSAR also performs based on conformation and alignment, which is absent in 3D QSAR, and it provides a site specific clue for designing new chemical entities. It outperforms the 2D QSAR where whole molecules are taken to consider the biological activity variation, but not the molecular fragments. Thus, the contribution of chemical variations against the biological activity with common lead molecules are less informative. Moreover the descriptor calculation is fast, as well as allowing ease of interpretation of the results.

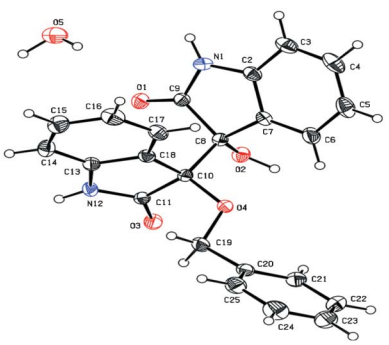
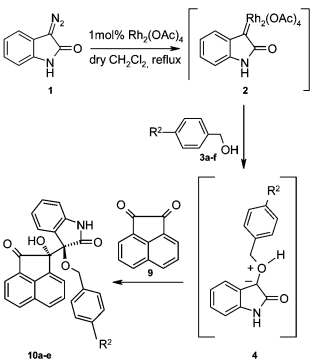


Fig. 1 ORTEP diagram of compound **6a**.



Scheme 4 Synthesis of 2-oxindolyl-2-hydroxyacenapthylen-1-ones **10a–e**.

Table 2 Synthesis of 2-oxindolyl-2-hydroxyindan-1,3-diones **8a–e**

Entry	Alcohol (**3**)	R^2	Product (**8**)[a]	Time (min)	Yield (%)[b]
1	**3a**	H	**8a**	70	85
2	**3b**	OMe	**8b**	70	88
3	**3c**	Me	**8c**	70	87
4	**3d**	Cl	**8d**	80	85
5	**3e**	Br	**8e**	80	80

[a] The products were characterized by IR, NMR, mass and elemental analysis. [b] Isolated yield after recrystallization.

In the case of *Escherichia coli*, compounds **6b**, **6d** and **6e** are the most active and **6a**, **6c** and **10e** are the least active. The GQSAR equation developed for *E. coli* and the corresponding statistics are given below:

Activity = $-0.0228–0.4653(\pm 0.0227)$*$R^1$-QMDipoleY + $54.9364(\pm 10.5006)$*R^1-Average +ve potential

$n = 15$, $r^2 = 0.76$, r^2 adj = 0.74, $q^2 = 0.69$, F-test = 18.83, r^2 se = 0.1164, q^2 se = 0.131

The equation consists of two descriptors, namely QMdipoleY and Average +ve potential. QMdipoleY contributes negatively to the activity. This descriptor deals with the strength and orientation of a molecule in an electrostatic field and is a function of the polarity. So less polar compounds will have higher activity. Similar results have been reported by Tantitame *et al.*, that less polar diastereomers exert better antibacterial activity when compared to the more polar diastereomers.[25] Other researchers have also found the contribution of the descriptor, QMDipoleZ, towards the antibacterial activity.[26] Average +ve potential descriptor belongs to the electrostatic subclass and it is defined as the average of the total +ve electrostatic potential on van der Waals surface area of the molecule and it showed a positive contribution towards the activity. The membranes of *Escherichia coli* contain negatively charged phosphate groups, which can attract more of the positively charged molecules.[27] So the positively charged molecules can bind easily to the wall of *Escherichia coli* and exhibit higher activity than negatively charged molecules. Due to the positive contribution of the average +ve potential and hydrophobicity of the molecules, this group of compounds are called hydrophobic cations, which have good permeability to the cell and organelles reported by Lewis.[28] Both the descriptors relate to the properties of the functional groups in the R^1 position. Fig. 2 shows the parity plot between the observed and the predicted antibacterial activities against *Escherichia coli* for training and test set compounds. All of the predictions lie within the 99% confidence limits. Table 5 lists the numerical values of the descriptors used in the training and test set of *Escherichia coli.*

Table 3 Synthesis of 2-oxindolyl-2-hydroxyacenaphthylen-1-ones **10a–e**

Entry	Alcohol (**3**)	R^2	Product (**10**)[a]	Time (min)	Yield (%)[b]
1	**3a**	H	**10a**	75	81
2	**3b**	OMe	**10b**	75	84
3	**3c**	Me	**10c**	75	82
4	**3d**	Cl	**10d**	80	81
5	**3e**	Br	**10e**	80	83

[a] The products were characterized by IR, NMR, mass and elemental analysis. [b] Isolated yield after recrystallization.

In the case of *Pseudomonas aeruginosa*, compounds **6b**, **6d** and **6e** are the most active and compounds **6a**, **6c** and **10e** are the least active. The GQSAR equation for *Pseudomonas aeruginosa* and the corresponding statistics are given below:

Activity = $-1.1765–1.1353(\pm 0.2523)$*$R^1$-QMDipoleY + $0.1912(\pm 0.0195)$* R^1-4PathCount

$n = 15$, $r^2 = 0.74$, r^2 adj = 0.72, $q^2 = 0.64$, F-test = 17.27, r^2 se = 0.22, q^2 se = 0.27

R^1-QMDipoleY descriptor contributes negatively to the activity. As mentioned before, hydrophobic substitution at the R^1 position favours activity. R^1-4PathCount belongs to the topological descriptor and shows a positive contribution towards the activity. It indicates the total number of fragments of fourth order (four bond path) in a compound.

This group of descriptors describes the connectivity of the atoms within the molecule and also explains its branching and flexibility (or rigidity).[29] An increase in branching increases the hydrophobicity.[30] Khan *et al.* also showed the positive contribution of hydrophobicity of the molecule with the antibacterial activity against *Pseudomonas aeruginosa*.[31] Fig. 3 shows the parity plot between the observed and the predicted antibacterial activities against *Pseudomonas aeruginosa* for training and test set compounds. All of the predictions lie within the 99% confidence limits. Table 6 lists the numerical values of the descriptors used in the GQSAR.

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

Activity = $-1.2257–1.0943(\pm 0.1655)$*$R^1$-QMDipoleY + $5.3711(\pm 1.3019)$*R^1-chiV5

$n = 15$, $r^2 = 0.66$, r^2 adj = 0.63, $q^2 = 0.54$, F-test = 11.63, r^2 se = 0.24, q^2 se = 0.28

Once again we find R^1-QMDipoleY as one of the descriptors in the equation. ChiV5 belongs to the topological descriptor in the sub-class of atomic valence connectivity index. It shows a positive contribution towards the activity, which indicates that hydrophobic substitution at R^1 position favours activity. Fig. 4 shows the parity plot between the observed and the predicted antibacterial activities against *Proteus vulgaris* for training and test set compounds.

All of the predictions lie within the 99% confidence limits. Table 7 lists the numerical values of the descriptors.

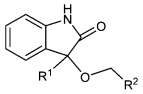
In the case of *Salmonella typhimurium*, compounds **6b**, **6d**, **6e** and **8b** are the most active and compounds **6a**, **6c** and **8e** are the least active. The GQSAR equation for *Salmonella typhimurium* and the corresponding statistics are given below:

Activity = $0.4156–0.7612(\pm 0.1424)$*$R^1$-QMDipoleY + $0.1119(\pm 0.0151)$*R^1-SssOE-index

$N = 15$, $r^2 = 0.85$, r^2 adj = 0.84, $q^2 = 0.79$, F-test = 34.17, r^2 se = 0.13, q^2 se = 0.15

R^1-SssOE-index is the electrotopological index and is defined as the number of oxygen atoms connected with two single bonds.[32] Among the active compounds, **6b** and **8b** have a SssOE-index of 4.19, which is relatively high (other

Table 4 Oxindoles synthesized with different substitutions and their antibacterial activities



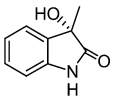
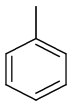
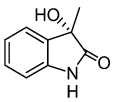
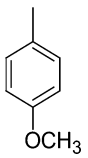
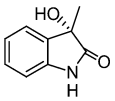
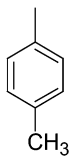
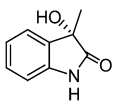
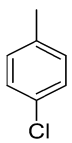
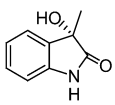
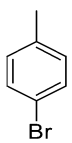
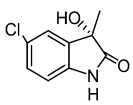
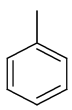
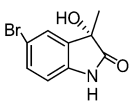
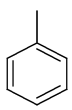
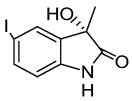
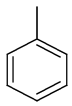
Compound no.	Substitutions used in GQSAR		Antibacterial activity (MIC in μM)				
	R^1	R^2	*S. aureus*	*P. aeruginosa*	*E. coli*	*P. vulgaris*	*S. typhi*
6a			0.647	1.294	0.323	1.294	0.647
6b			0.075	0.038	0.038	0.075	0.038
6c			1.249	1.249	0.312	2.497	0.624
6d			0.149	0.074	0.074	0.149	0.074
6e			0.067	0.067	0.034	0.067	0.034
6f			0.594	0.594	0.149	1.188	0.297
6g			0.537	0.537	0.134	0.537	0.269
6h			0.488	0.976	0.244	0.976	0.244

Table 4 (*Continued*)

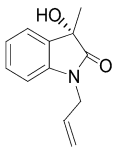
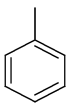
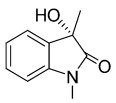
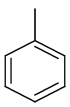
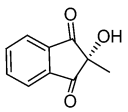
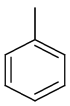
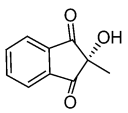
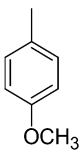
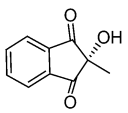
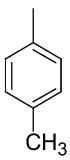
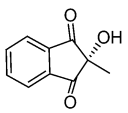
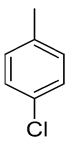
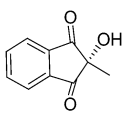
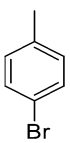
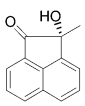
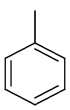
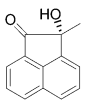
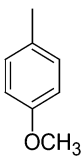
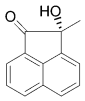
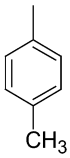
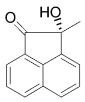
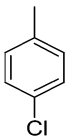
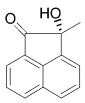
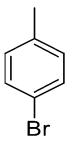
Compound no.	Substitutions used in GQSAR		Antibacterial activity (MIC in μM)				
	R^1	R^2	*S. aureus*	*P. aeruginosa*	*E. coli*	*P. vulgaris*	*S. typhi*
6i			0.586	0.586	0.147	0.586	0.293
6j			0.624	0.624	0.156	0.624	0.312
8a			0.313	0.626	0.156	0.313	0.313
8b			0.291	0.291	0.146	0.291	0.146
8c			0.605	0.605	0.076	0.605	0.302
8d			0.288	0.288	0.144	0.288	0.288
8e			0.523	0.523	0.131	0.523	0.523
10a			1.186	1.186	0.148	1.186	0.297
10b			0.277	0.554	0.138	1.107	0.277

Table 4 (*Continued*)

Compound no.	Substitutions used in GQSAR		Antibacterial activity (MIC in μM)				
	R^1	R^2	*S. aureus*	*P. aeruginosa*	*E. coli*	*P. vulgaris*	*S. typhi*
10c			0.287	0.574	0.144	0.574	0.144
10d			0.548	1.097	0.137	1.097	0.548
10e			0.999	1.999	0.250	0.999	0.500

compounds has zero) compared to other compounds except compound **10b**. The latter also had a SssOE-index as 4.19 and it showed moderate activity. Fig. 5 shows the parity plot between the observed and the predicted antibacterial activities against *Salmonella typhimurium* for training and test set compounds. All of the predictions lie within the 99% confidence limits. Table 8 lists the numerical values of these descriptors.

In the case of *Staphylococcus aureus*, compounds **6b**, **6d** and **6e** are the most active and **6c**, **10a** and **10e** are the least active. The GQSAR equation for *Staphylococcus aureus* and its corresponding statistics are given below:

Activity = $-1.0378–0.6124(\pm 0.1928)$*$R^1$-QMDipoleY + $1.5986(\pm 0.3018)$*R^1-chi5

$N = 15$, $r^2 = 0.73$, r^2 adj = 0.71, $q^2 = 0.62$, F-test = 16.37, r^2 se = 0.17, q^2 se = 0.20

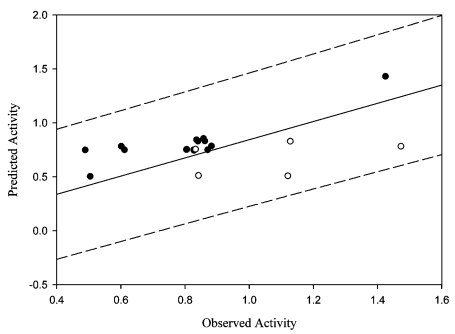


Fig. 2 A parity plot between the observed and the predicted antibacterial activities against *Escherichia coli* for training and test set compounds with 99% prediction bands (closed circles – training set; open circle – test set).

Chi5 belongs to the class of topological descriptors and it describes the connectivity of the molecule. This descriptor showed a positive contribution to the activity. An increase in the connectivity will increase the hydrophobicity of the molecule. Fig. 6 shows the parity plot between the observed and the predicted antibacterial activities against *Staphylococcus aureus* for training and test set compounds. All of the predictions lie within the 99% confidence limits. Table 9 lists the numerical values of the descriptors used in the training set and test set of *Staphylococcus aureus.*

Table 10 shows the percentage contribution of the descriptors used in GQSARs for all of the microorganisms. A dipole of the substituent group in the R^1 position appears to contribute negatively in all of the regression equations. Our previous research also showed the contribution of dipole moment towards the antibacterial activity of isoxazolidine derivatives against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.[33]

This method offers several advantages, such as a high yield and simple experimental and isolation procedures, making it an efficient route to the synthesis of 3-bisoxindoles, 2-oxindolyl-2-hydroxyindan-1,3-diones and 2-oxindolyl-2-hydroxyacenaphthylen-1-ones, which are important compounds in organic and medicinal chemistry.

Conclusion

In conclusion, we have demonstrated the first example of rhodium(II)-catalyzed one-pot, multicomponent reaction of 3-diazooxindole, benzyl alcohols and isatin or ninhydrin or acenaphthenequinone to synthesize 3-bisoxindoles, 2-oxindolyl-2-hydroxyindan-1,3-diones and 2-oxindolyl-2-hydroxyacenaphthylen-1-ones, respectively with adjacent tetra-substituted carbon

Table 5 The numerical values of the observed and predicted activity and descriptors used in GQSAR for *Escherichia coli*

Compound	Observed activity	R^1-QMDipoleY	R^1-Average +ve potential	Predicted activity
Training set				
6a	0.490	−0.118	0.013	0.745
6b	1.426	−0.585	0.021	1.428
6c	0.506	0.167	0.011	0.500
6f	0.828	−0.119	0.013	0.747
6g	0.872	−0.119	0.013	0.746
6h	0.613	−0.119	0.013	0.746
6j	0.807	−0.117	0.013	0.751
8a	0.806	−0.115	0.013	0.749
8b	0.837	0.250	0.018	0.840
8d	0.842	0.160	0.017	0.827
8e	0.884	0.143	0.016	0.782
10a	0.829	−0.112	0.013	0.742
10b	0.859	0.435	0.020	0.852
10d	0.863	0.157	0.017	0.828
10e	0.602	0.141	0.016	0.780
Test set				
6d	1.129	0.164	0.017	0.825
6e	1.474	0.148	0.016	0.779
6i	0.834	−0.118	0.013	0.753
8c	1.122	0.164	0.011	0.505
10c	0.843	0.157	0.011	0.508

centres. We suggest that the reaction occurs through a metal-associated alcoholic oxonium ylide intermediate and is followed by a nucleophilic addition to electrophiles, such as isatin, ninhydrin and acenaphthenequinone. The high electrophilicity and rigid cyclic conformation of isatin, ninhydrin and acenaphthenequinone are responsible for such a high chemo- and diastereoselectivity. Antibacterial activities of the synthesised oxindoles against five Gram +ve and Gram –ve organisms were evaluated using microdilution method. Compounds **6b**, **6d** and **6e** were found to be the most active against all of the five organisms. Group-based QSAR has been developed for all five antibacterial activities. The statistical parameters were found to be in an accepted range. Among the groups in the R^1 and R^2 position, the descriptor on R^1 is correlated with all of the antibacterial activities, which suggests the importance of the R^1 group. It shows that electrostatic (QMDipoleY, SssOE and average +ve potential) (acts upon membrane integrity and permeability) and, hydrophobic (chiV5, chi5 and 4PathCount) (for the penetration of the drug through the membrane and the retention of the drug within bacterial cell) interactions are important contributions for the antibacterial activity. Other researchers have also found the importance of electrostatic and lipophilicity towards the antibacterial activities.[34]

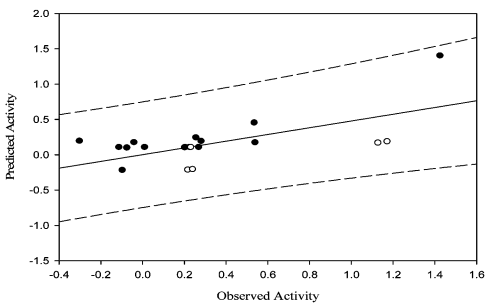


Fig. 3 A parity plot between the observed and the predicted antibacterial activities against *Pseudomonas aeruginosa* for training and test set compounds with 99% prediction bands (closed circles – training set; open circle – test set).

Experimental section

General

All of the chemicals required for determining the biological activities were purchased from Sigma-Aldrich (St Louis, MO, USA), Himedia (Mumbai, India) and SRL (Mumbai, India) and the five bacterial strains (*S. aureus* NCIM5021, *E. coli* NCIM 2931, *P. vulgaris* NCIM 2813, *S. typhi* NCIM 2501 and *P. aeruginosa* NCIM 5029) were purchased from National Chemical Laboratory, Pune, India. VLifemds3.5 is a software package for computer aided drug discovery (VLife Sciences Technologies Pvt. Ltd., Pune, India, http://www.vlifesciences.com) and it is used for molecular modelling. IR measurements were done as KBr pellets for solids using a Perkin Elmer Spectrum RXI FT-IR. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded as DMSO-d_6 solution for all compounds with TMS as an internal standard on a JEOL instrument. Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), multiplet (m), and broad (br). The mass of the compounds was analyzed using an Electrospray Ionisation Method with Thermo Finnigan Mass spectrometer. Melting points were determined in capillary tubes and are uncorrected. Elemental analyses were recorded using a Thermo Finnigan FLASH EA 1112 CHN analyzer. Analytical TLC was performed on precoated plastic sheets of silica gel G/UV-254 of 0.2mm thickness (Macherey-Nagel). To facilitate the QSAR, the observed activity (Minimum Inhibitory Concentration against the three microorganisms calculated as μg

Table 6 The numerical values of the observed and predicted activity and descriptors used in GQSAR for *Pseudomonas aeruginosa*

Compound	Observed activity	R^1-QMDipoleY	R^1-4PathCount	Predicted activity
Training set				
6a	−0.112	−0.118	6	0.105
6b	1.426	−0.585	10	1.400
6c	−0.096	0.167	6	−0.219
6f	0.226	−0.119	6	0.106
6g	0.270	−0.119	6	0.106
6h	0.011	−0.119	6	0.106
6j	0.205	−0.117	6	0.104
8a	0.203	−0.115	6	0.102
8b	0.536	0.250	10	0.452
8d	0.540	0.160	8	0.172
8e	0.282	0.143	8	0.191
10a	−0.074	−0.112	6	0.098
10b	0.257	0.435	10	0.242
10d	−0.040	0.157	8	0.175
10e	−0.301	0.141	8	0.193
Test set				
6d	1.129	0.164	8	0.167
6e	1.173	0.148	8	0.186
6i	0.232	−0.118	6	0.105
8c	0.218	0.164	6	−0.215
10c	0.241	0.157	6	−0.207

ml^{-1}) was converted to −log (μM). Spectral data is given in the supporting information†.

Antibacterial evaluation

In vitro antibacterial activity (MIC) of the oxindole derivatives were determined by microdilution broth assay method with modifications, as reported by Sarker *et al.*, using resazurin as an indicator.[35] Muller hinton broth was used to grow the bacterial strains to a final inoculum size of 5 × 105 CFU mL^{-1}. The compounds were dissolved in absolute ethanol to a concentration of 10 mg mL^{-1}.[36] These serially diluted solutions were added to successive wells in a 96-well microtiter plate and incubated with the organisms for 18 h at 37 °C. Growth and sterility controls were maintained during the experiment. Compounds serially diluted with ethanol were also kept in the 96-well microtiter plates (uninoculated dilution) to determine if they precipitated out during the course of the experiments. A blank array with ethanol alone and its effect on the inhibition of the microorganism was also studied. Ten microliters of 0.01% resazurin solution was added and incubated for 2 h. The effect of ethanol on the growth of the microorganism was also studied. The color change was assessed visually, with the highest dilution remaining blue (inhibition of growth) indicating the minimum inhibitory concentration. A change in color from blue to pink shows the growth of the organism.

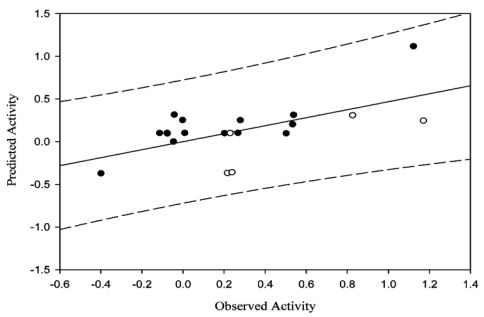


Fig. 4 A parity plot between the observed and the predicted antibacterial activities against *Proteus vulgaris* for training and test set compounds with 99% prediction bands (closed circles – training set; open circle – test set).

Modelling studies

Twenty compounds were synthesized, which have the oxindole as the common template, while the functional groups at the R^1 and R^2 positions are different. The MIC (μM) data of oxindoles

Table 7 The numerical values of the observed and predicted activity and descriptors used in GQSAR for *Proteus vulgaris*

Compound	Observed activity	R^1-QMDipoleY	R^1-chiV5	Predicted activity
Training set				
6j	0.205	−0.117	0.222	0.096
8a	0.505	−0.115	0.222	0.094
8b	0.536	0.250	0.316	0.200
8d	0.540	0.160	0.318	0.310
8e	0.282	0.143	0.304	0.249
10a	−0.074	−0.112	0.222	0.091
10b	−0.044	0.435	0.316	−0.002
10d	−0.040	0.157	0.318	0.313
6a	−0.112	−0.118	0.222	0.097
10e	0.000	0.141	0.304	0.250
6b	1.125	−0.585	0.316	1.114
6c	−0.397	0.167	0.192	−0.375
6f	−0.075	−0.119	0.222	0.098
6g	0.270	−0.119	0.222	0.098
6h	0.011	−0.119	0.222	0.098
Test set				
8c	0.218	0.164	0.192	−0.371
10c	0.241	0.157	0.192	−0.363
6d	0.828	0.164	0.318	0.305
6e	1.173	0.148	0.304	0.243
6i	0.232	−0.118	0.222	0.097

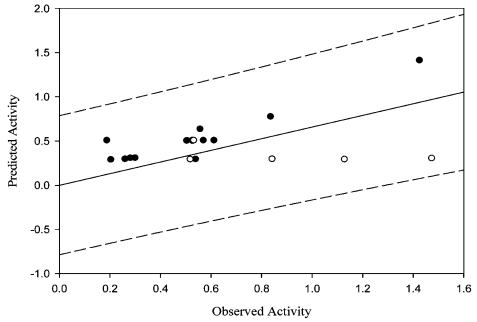


Fig. 5 A parity plot between the observed and the predicted antibacterial activities against *Salmonella typhimurium* for training and test set compounds with 99% prediction bands (closed circles – training set; open circle – test set).

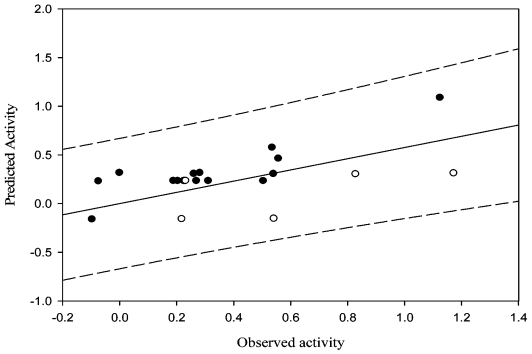


Fig. 6 A parity plot between the observed and the predicted antibacterial activities against *Staphylococcus aureus* for training and test set compounds with 99% prediction bands (closed circles – training set; open circle – test set).

listed in Table 4 was converted to −log (μM) for the development of the QSAR. Twenty of these oxindoles were sketched using the Vlife mds 3.5 software and their minimum energy conformations were determined using MMFF force field.[37] Three hundred and seventy four descriptors relating to spatial, electronic, thermodynamic, conformational, topological, information-content, quantum mechanical and structural properties of the substituent groups in the R^1 and R^2 position for all of the compounds were calculated. Several references deal in detail with these descriptors.[29,38,39] The total twenty molecules were divided into training and test sets. The former consists of fifteen molecules, which are used for the development of the GQSAR equation and the latter consisting of five molecules (compounds **6d**, **6e**, **6i**, **8c** and **10c**), which were selected at random were used for the validation of these models. This technique is known as an external validation. The developed GQSAR is also validated by calculating several statistical parameters, such as r^2, r^2 adj, q^2, *F*-ratio and standard error.[40] This procedure is known as the internal validation method. A Genetic Algorithm technique (GFA) is used to develop the best GQSAR equation relating the descriptors with the activity. The GFA technique is useful when one has a large pool of descriptors, but limited data, where the challenge is to select the best set of descriptors. Other researchers have also used the GFA technique effectively to develop the QSAR models.[41]

Representative procedure for preparation of compounds 6a–j

To a refluxing dry CH_2Cl_2 solution of $Rh_2(OAc)_4$ (1 mol%), benzyl alcohol **3** (0.60 mmol) and isatin **5** (0.50 mmol) under argon atmosphere, 3-diazo-1,3-dihydro-indol-2-one **1** (0.60 mmol) in dry dichloromethane was added dropwise (approximately 10 ml in 60 min). After the addition was completed, the reaction mixture was allowed to reflux for 30–40 min until the disappearance of the starting materials, as determined by TLC, and cooled to room temperature. The solid formed in the reaction mixture was filtered, dried and recrystallized from

Table 8 The numerical values of the observed and predicted activity and descriptors used in GQSAR for *Salmonella typhimurium*

Compound	Observed activity	R^1-QMDipoleY	R^1-OxygensCount	Predicted activity
Training set				
6j	0.506	−0.117	0	0.505
8a	0.505	−0.115	0	0.503
8b	0.837	0.250	1	0.775
8d	0.540	0.160	0	0.294
8e	0.282	0.143	0	0.307
10a	0.528	−0.112	0	0.501
10b	0.558	0.435	1	0.634
10d	0.261	0.157	0	0.296
6a	0.189	−0.118	0	0.506
6b	1.426	−0.585	1	1.411
6c	0.205	0.167	0	0.288
6f	0.527	−0.119	0	0.506
6g	0.571	−0.119	0	0.506
6h	0.613	−0.119	0	0.506
10e	0.301	0.141	0	0.308
Test set				
6d	1.129	0.164	0	0.291
6e	1.474	0.148	0	0.303
6i	0.533	−0.118	0	0.506
8c	0.519	0.164	0	0.291
10c	0.843	0.157	0	0.296

Table 9 The numerical values of the observed and predicted activity and descriptors used in GQSAR for *Staphylococcus aureus*

Compound	Observed activity	R^1-chi5	R^1-QMDipoleY	Predicted activity
Training set				
6a	0.189	0.75	−0.12	0.234
6b	1.125	1.11	−0.58	1.087
6c	−0.096	0.61	0.17	−0.161
6f	0.226	0.75	−0.12	0.234
6g	0.270	0.75	−0.12	0.234
6h	0.312	0.75	−0.12	0.234
6j	0.205	0.75	−0.12	0.233
8a	0.505	0.75	−0.12	0.232
8b	0.536	1.11	0.25	0.576
8d	0.540	0.90	0.16	0.305
8e	0.282	0.90	0.14	0.315
10a	−0.074	0.75	−0.11	0.230
10b	0.558	1.11	0.43	0.463
10d	0.261	0.90	0.16	0.306
10e	0.000	0.90	0.14	0.316
Test set				
6d	0.828	0.90	0.16	0.302
6e	1.173	0.90	0.15	0.312
6i	0.232	0.75	−0.12	0.234
8c	0.218	0.61	0.16	−0.159
10c	0.542	0.61	0.16	−0.155

ethanol to obtain the pure product and appropriate isolated yield is shown in Table 1.

Representative procedure for the synthesis of 2-oxindolyl-2-hydroxyindan-1,3-diones (8a–e)

To a refluxing dry CH_2Cl_2 solution of $Rh_2(OAc)_4$ (1 mol%), benzyl alcohol **3** (0.60 mmol) and ninhydrin **7** (0.50 mmol) under argon atmosphere 3-diazo-1,3-dihydro-indol-2-one **1** (0.60 mmol) in dry dichloromethane was added dropwise (approximately 10 ml in 60 min). After the addition was completed, the reaction mixture was allowed to reflux for 10–20 min until the disappearance of starting materials, as determined by TLC, and was cooled in a refrigerator for about 60 min. The solid formed in the reaction mixture was filtered, dried and recrystallized from ethanol to obtain the pure product and the appropriate isolated yield is shown in Table 2.

Representative procedure for the synthesis of 2-oxindolyl-2-hydroxyacenaphthylen-1-ones (10a–e)

To a refluxing dry CH_2Cl_2 solution of $Rh_2(OAc)_4$ (1 mol%), benzyl alcohol **3** (0.60 mmol) and acenaphthenequinone **9** (0.50 mmol) under argon atmosphere, 3-diazo-1,3-dihydro-indol-2-one **1** (0.60 mmol) in dry dichloromethane was added dropwise (approximately 10 ml in 60 min). After the addition was completed, the reaction mixture was allowed to reflux for 15–20 min until the disappearance of the starting materials, as determined by TLC. The solid formed in the reaction mixture was filtered, dried and recrystallized from ethanol to obtain the pure product and the appropriate isolated yield is shown in Table 3.

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Table 10 The percentage contribution of the short-listed descriptors for developing GQSAR equation

Organism Name	Descriptor 1	% contribution of descriptor 1	Descriptor 2	% contribution of descriptor 2
Escherichia coli NCIM 2931	R^1-QMDipoleY	−40.72%	R^1-Average+vePotential	59.28%
Pseudomonas aeruginosa NCIM 5029	R^1-QMDipoleY	−46.63%	R^1-4PathCount	53.37%
Proteus vulgaris NCIM 2813	R^1-QMDipoleY	−49.61%	R^1-chiV5	50.39%
Salmonella typhimurium NCIM 2501	R^1-QMDipoleY	−44.55%	R^1-SssOE	55.45%
Staphylococcus aureus NCIM5021	R^1-QMDipoleY	−37.40%	R^1-chi5	62.60%

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